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(54) Title: DIAGNOSIS AND TREATMENT OF PHOSPHATASE OR KINASE-RELATED DISORDERS			
(57) Abstract The present invention relates to phosphatases and kinases, nucleic acids encoding such polypeptides, cells, tissues and animals containing such nucleic acids, antibodies to such polypeptides, assays utilizing such polypeptides, and methods relating to all of the foregoing. Methods for treatment, diagnosis, and screening are provided for phosphatase or kinase related diseases or conditions characterized by an abnormal interaction between a phosphatase or a kinase and its binding partner.			

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DESCRIPTIONDiagnosis And Treatment Of
Tyrosine Phosphatase-Related Disorders
And Related Methods

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Field Of The Invention

The present invention relates to protein tyrosine phosphatases. In particular, the invention concerns proteins we have named PTP04, SAD, PTP05, PTP10, ALP, and ALK-7, nucleotide sequences encoding these proteins, and various products and assay methods that can be used for identifying compounds useful for the diagnosis and treatment of various diseases and conditions related to these proteins, for example cell proliferative disorders.

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Background Of The Invention

The following description is provided to aid in understanding the invention but is not admitted to be prior art to the invention.

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Cellular signal transduction is a fundamental mechanism whereby external stimuli that regulate diverse cellular processes are relayed to the interior of cells. One of the key biochemical mechanisms of signal transduction involves the reversible phosphorylation of proteins, which enables regulation of the activity of mature proteins by altering their structure and function. The best characterized protein kinases in eukaryotes phosphorylate proteins on the alcohol moiety of serine, threonine and tyrosine residues. These kinases largely fall into two groups, those specific for phosphorylating serines and threonines, and those specific for phosphorylating tyrosines.

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The phosphorylation state of a given substrate is also regulated by a class of proteins responsible for removal of the

phosphate group added to a given substrate by a protein kinase. The protein phosphatases can also be classified as being specific for either serine/threonine or tyrosine. The known enzymes can be divided into two groups - receptor and non-receptor type proteins. Most receptor-type protein tyrosine phosphatases (RPTPs) contain two conserved catalytic tyrosine phosphatase domains each of which encompasses a segment of 240 amino acid residues (Saito et al, Cell Growth and Diff. 2:59-65, 1991). The RPTPs can be subclassified further based upon the amino acid sequence diversity of their extracellular domains (Saito, et al, supra; Krueger, et al, Proc. Natl. Acad. Sci. USA 89:7417-7421, 1992). Alignment of primary peptide sequences of both types of known PTPases shows some sequence consensus in catalytic domains and has made it possible to identify cDNAs encoding proteins with tyrosine phosphate activity via the polymerase chain reaction (PCR).

Many kinases and phosphatases are involved in regulatory cascades wherein their substrates may include other kinases and phosphatases whose activities are regulated by their phosphorylation state. Ultimately the activity of some downstream effector is modulated by phosphorylation resulting from activation of such a pathway.

It is well established that the abnormal or inappropriate activity of tyrosine kinases and/or tyrosine phosphatases plays a role in a variety of human disorders including cell proliferative disorders such as cancer, fibrotic disorders, disorders of the immune system and metabolic disorders such as diabetes. A need, therefore, exists to identify new tyrosine kinases and phosphatases as a first step in understanding a disease process and the subsequent identification of therapeutic treatments for the disorder.

Summary Of The Invention

The present invention concerns PTP04, SAD, PTP05, PTP10, Alp, and ALK-7 polypeptides, nucleic acids encoding such polypeptides, cells, tissues and animals containing such nucleic acids, antibodies to the polypeptides, assays utilizing the polypeptides, and methods relating to all of the foregoing.

A first aspect of the invention features an isolated, enriched, or purified nucleic acid molecule encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide.

By "isolated" in reference to nucleic acid is meant a polymer of 14, 17, 21 or more nucleotides conjugated to each other, including DNA or RNA that is isolated from a natural source or that is synthesized. The isolated nucleic acid of the present invention is unique in the sense that it is not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular (i.e., chromosomal) environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only nucleotide sequence present, but that it is essentially free (about 90 - 95% pure at least) of non-nucleotide material naturally associated with it and thus is meant to be distinguished from isolated chromosomes.

By the use of the term "enriched" in reference to nucleic acid is meant that the specific DNA or RNA sequence constitutes a significantly higher fraction (2 - 5 fold) of the total DNA or RNA present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other DNA or RNA present, or by a preferential increase in the amount of the specific DNA or RNA sequence, or by a combination of the two.

However, it should be noted that "enriched" does not imply that there are no other DNA or RNA sequences present, just that the relative amount of the sequence of interest has been significantly increased. The term "significant" here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other nucleic acids of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there is no DNA or RNA from other sources. The other source DNA may, for example, comprise DNA from a yeast or bacterial genome, or a cloning vector such as pUC19. This term distinguishes the sequence from naturally occurring enrichment events, such as viral infection, or tumor type growths, in which the level of one mRNA may be naturally increased relative to other species of mRNA. That is, the term is meant to cover only those situations in which a person has intervened to elevate the proportion of the desired nucleic acid.

It is also advantageous for some purposes that a nucleotide sequence be in purified form. The term "purified" in reference to nucleic acid does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, e.g., in terms of mg/mL). Individual clones isolated from a cDNA library may be purified to electrophoretic homogeneity. The claimed DNA molecules obtained from these clones can be obtained directly from total DNA or from total RNA. The cDNA clones are not naturally occurring, but rather are preferably obtained via manipulation of a partially purified naturally occurring substance (messenger RNA). The construction of a cDNA library from mRNA involves the creation of a synthetic substance (cDNA) and pure

individual cDNA clones can be isolated from the synthetic library by clonal selection of the cells carrying the cDNA library. Thus, the process which includes the construction of a cDNA library from mRNA and isolation of distinct cDNA clones yields an approximately 10^6 -fold purification of the native message. Thus, purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. The term is also chosen to distinguish clones already in existence which may encode PTP04, SAD, PTP05, PTP10, ALP, or Alk-7 but which have not been isolated from other clones in a library of clones. Thus, the term covers clones encoding PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 which are isolated from other non-PTP04, non-SAD, non-PTP05, non-PTP10, non-ALP, or non-ALK-7 clones.

A PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide can be encoded by a full-length nucleic acid sequence or any portion of the full-length nucleic acid sequence. In preferred embodiments the isolated nucleic acid comprises, consists essentially of, or consists of a nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8, a nucleic acid sequence that hybridizes to the nucleic acid sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8 or a functional derivative (as defined below) of either. The nucleic acid may be isolated from a natural source by cDNA cloning or subtractive hybridization; the natural source may be mammalian (human) blood, semen, or tissue and the nucleic acid may be synthesized by the triester or other method or by using an automated DNA synthesizer.

The term "hybridize" refers to a method of interacting a nucleic acid sequence with a DNA or RNA molecule in solution or

on a solid support, such as cellulose or nitrocellulose. If a nucleic acid sequence binds to the DNA or RNA molecule with high affinity, it is said to "hybridize" to the DNA or RNA molecule. The strength of the interaction between the probing
5 sequence and its target can be assessed by varying the stringency of the hybridization conditions. Various low or high stringency hybridization conditions may be used depending upon the specificity and selectivity desired. Stringency is controlled by varying salt or denaturant concentrations.

10 As a general guideline, high stringency conditions (hybridization at 50-65 °C, 5X SSPC, 50% formamide, wash at 50-65 °C, 0.5X SSPC) can be used to obtain hybridization between nucleic acid sequences having regions which are greater than about 90% complementary. Low stringency conditions
15 (hybridization at 35-37 °C, 5X SSPC, 40-45% formamide, wash at 42 °C SSPC) can be used so that sequences having regions which are greater than 35-45% complementarity will hybridize to the probe. These conditions only represent examples of stringency conditions and those skilled in the art recognize that these
20 conditions may be changed depending on the particular mode of practice. Further examples of hybridization conditions are shown in the examples below. Those skilled in the art will recognize how such conditions can be varied to vary specificity and selectivity. Under highly stringent hybridization
25 conditions only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having one or two mismatches out of 20 contiguous nucleotides.

30 In yet other preferred embodiments the nucleic acid is an isolated conserved or unique region, for example those useful for the design of hybridization probes to facilitate identification and cloning of additional polypeptides, or for the

design of PCR probes to facilitate cloning of additional polypeptides.

By "conserved nucleic acid regions", it is meant regions present on two or more nucleic acids encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide, to which a particular nucleic acid sequence can hybridize under lower stringency conditions. Examples of lower stringency conditions suitable for screening for nucleic acids encoding PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptides are provided in Abe, et al. J. Biol. Chem. 19:13361 (1992). Preferably, conserved regions differ by no more than 5 out of 20 contiguous nucleotides.

By "unique nucleic acid region" it is meant a sequence present in a full length nucleic acid coding for a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide that is not present in a sequence coding for any other known naturally occurring polypeptide. Such regions preferably comprise 14, 17, 21 or more contiguous nucleotides present in the full length nucleic acid encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide. In particular, a unique nucleic acid region is preferably of human origin.

The invention also features a nucleic acid probe for the detection of a nucleic acid encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide in a sample. The nucleic acid probe contains nucleic acid that will hybridize specifically to a sequence of at least 14, preferably 17, 20 or 22, contiguous nucleotides set forth in SEQ ID NO:1 or a functional derivative thereof. The probe is preferably at least 14, 17 or more bases in length and selected to hybridize specifically to a unique region of a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 encoding nucleic acid.

In preferred embodiments the nucleic acid probe hybridizes to nucleic acid encoding at least 14 contiguous amino acids of

the full-length sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8 or a functional derivative thereof. Various low or high stringency hybridization conditions may be used depending upon the specificity and selectivity desired. Under highly stringent hybridization conditions only highly complementary nucleic acid sequences hybridize. Preferably, such conditions prevent hybridization of nucleic acids having 1 or 2 mismatches out of 20 contiguous nucleotides.

Methods for using the probes include detecting the presence or amount of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 RNA in a sample by contacting the sample with a nucleic acid probe under conditions such that hybridization occurs and detecting the presence or amount of the probe bound to PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 RNA. The nucleic acid duplex formed between the probe and a nucleic acid sequence coding for a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide may be used in the identification of the sequence of the nucleic acid detected (for example see, Nelson et al., in Nonisotopic DNA Probe Techniques, p. 275 Academic Press, San Diego (Kricka, ed., 1992)). Kits for performing such methods may be constructed to include a container means having disposed therein a nucleic acid probe.

The invention also features recombinant nucleic acid, preferably in a cell or an organism. The recombinant nucleic acid may contain a sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8 or a functional derivative thereof and a vector or a promoter effective to initiate transcription in a host cell. The recombinant nucleic acid can alternatively contain a transcriptional initiation region functional in a cell, a sequence complimentary to an RNA sequence encoding a

PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide and a transcriptional termination region functional in a cell.

Another aspect of the invention features an isolated, enriched, or purified PTP04, SAD, PTP05, PTP10, ALP, or ALK-7
5 polypeptide.

By "PTP04 polypeptide" it is meant an amino acid sequence substantially similar to the sequence shown in SEQ ID NO:9, or fragments thereof. By "SAD polypeptide" it is meant an amino acid sequence substantially similar to the sequence shown in
10 SEQ ID NO:10, or fragments thereof. By "PTP05 polypeptide" or "PTP10 polypeptide" it is meant an amino acid sequence substantially similar to the sequence shown in SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, or SEQ ID NO:14, or fragments thereof. By "ALP polypeptide" it is meant an amino acid
15 sequence substantially similar to the sequence shown in SEQ ID NO:15, or fragments thereof. By "ALK-7 polypeptide" it is meant an amino acid sequence substantially similar to the sequence shown in SEQ ID NO:16, or fragments thereof. Two substantially similar sequences will preferably have at least
20 90% identity (more preferably at least 95% and most preferably 99-100%) to each other.

By "identity" is meant a property of sequences that measures their similarity or relationship. Identity is measured by dividing the number of identical residues in the
25 two sequences by the total number of residues and multiplying the product by 100. Thus, two copies of exactly the same sequence have 100% identity, but sequences that are less highly conserved and have deletions, additions, or replacements have a lower degree of identity. Those skilled in the art will
30 recognize that several computer programs are available for determining sequence identity.

By "isolated" in reference to a polypeptide is meant a polymer of 6, 12, 18 or more amino acids conjugated to each

other, including polypeptides that are isolated from a natural source or that are synthesized. The isolated polypeptides of the present invention are unique in the sense that they are not found in a pure or separated state in nature. Use of the term "isolated" indicates that a naturally occurring sequence has been removed from its normal cellular environment. Thus, the sequence may be in a cell-free solution or placed in a different cellular environment. The term does not imply that the sequence is the only amino acid chain present, but that it is essentially free (about 90 - 95% pure at least) of material naturally associated with it.

By the use of the term "enriched" in reference to a polypeptide it is meant that the specific amino acid sequence constitutes a significantly higher fraction (2 - 5 fold) of the total of amino acids present in the cells or solution of interest than in normal or diseased cells or in the cells from which the sequence was taken. This could be caused by a person by preferential reduction in the amount of other amino acids present, or by a preferential increase in the amount of the specific amino acid sequence of interest, or by a combination of the two. However, it should be noted that "enriched" does not imply that there are no other amino acid sequences present, just that the relative amount of the sequence of interest has been significantly increased. The term significant here is used to indicate that the level of increase is useful to the person making such an increase, and generally means an increase relative to other amino acids of about at least 2 fold, more preferably at least 5 to 10 fold or even more. The term also does not imply that there is no amino acid from other sources. The other source amino acid may, for example, comprise amino acid encoded by a yeast or bacterial genome, or a cloning vector such as pUC19. The term is meant to cover only those

situations in which a person has intervened to elevate the proportion of the desired nucleic acid.

It is also advantageous for some purposes that an amino acid sequence be in purified form. The term "purified" in reference to a polypeptide does not require absolute purity (such as a homogeneous preparation); instead, it represents an indication that the sequence is relatively purer than in the natural environment (compared to the natural level this level should be at least 2-5 fold greater, e.g., in terms of mg/mL).

Purification of at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. The substance is preferably free of contamination at a functionally significant level, for example 90%, 95%, or 99% pure.

In another aspect the invention features an isolated, enriched, or purified PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide fragment.

By "a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide fragment" it is meant an amino acid sequence that is less than the full-length PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 amino acid sequence shown in SEQ ID NO:2. Examples of fragments include PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 domains, PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 mutants and PTP04-, SAD-, PTP05-, PTP10-, ALP-, or ALK-7-specific epitopes.

By "a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain" it is meant a portion of the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide having homology to amino acid sequences from one or more known proteins wherein the sequence predicts some common function, interaction or activity. Well known examples of domains are the SH2 (Src Homology 2) domain (Sadowski, et al, Mol. Cell. Biol. 6:4396, 1986; Pawson and Schlessinger, Curr. Biol. 3:434, 1993), the SH3 domain (Mayer, et al, Nature 332:272, 1988; Pawson and Schlessinger, Curr.

Biol. 3:434, 1993), and pleckstrin (PH) domain (Ponting, TIBS 21:245, 1996; Haslam, et al, Nature 363:309, 1993), all of which are domains that mediate protein:protein interaction, and the kinase catalytic domain (Hanks and Hunter, FASEB J 9:576-595, 1995). Computer programs designed to detect such homologies are well known in the art. The relative homology is at least 20%, more preferably at least 30% and most preferably at least 35%.

By "a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 mutant" it is meant a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide which differs from the native sequence in that one or more amino acids have been changed, added or deleted. Changes in amino acids may be conservative or non-conservative. By "conservative" it is meant the substitution of an amino acid for one with similar properties such as charge, hydrophobicity, structure, etc. Examples of polypeptides encompassed by this term include, but are not limited to, (1) chimeric proteins which comprise a portion of a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide sequence fused to a non-PTP04, a non-SAD, a non-PTP05, a non-PTP10, a non-ALP, or a non-ALK-7 polypeptide sequence, for example a polypeptide sequence of hemagglutinin (HA), (2) PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 proteins lacking a specific domain, for example the catalytic domain, and (3) PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 proteins having a point mutation. A PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 mutant will retain some useful function such as, for example, binding to a natural binding partner, catalytic activity, or the ability to bind to a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 specific antibody (as defined below).

By "PTP04-, SAD-, PTP05-, PTP10-, ALP-, or ALK-7-specific epitope" it is meant a sequence of amino acids that is both antigenic and unique to PTP04, SAD, PTP05, PTP10, ALP, or ALK-

7. PTP04-, SAD-, PTP05-, PTP10-, ALP-, or ALK-7-specific epitope can be used to produce PTP04-, SAD-, PTP05-, PTP10-, ALP-, or ALK-7-specific antibodies, as more fully described below. Particularly preferred epitopes are shown in Examples 5 below.

By "recombinant PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide" it is meant to include a polypeptide produced by recombinant DNA techniques such that it is distinct from a naturally occurring polypeptide either in its location (e.g., present in a different cell or tissue than found in nature), purity or structure. Generally, such a recombinant polypeptide will be present in a cell in an amount different from that normally observed in nature.

In yet another aspect the invention features an antibody (e.g., a monoclonal or polyclonal antibody) having specific binding affinity to a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide or polypeptide fragment. By "specific binding affinity" is meant that the antibody binds to target polypeptide with greater affinity than it binds to other polypeptides under specified conditions. Antibodies or antibody fragments are polypeptides which contain regions that can bind other polypeptides. The term "specific binding affinity" describes an antibody that binds to a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide with greater affinity than it binds to other polypeptides under specified conditions.

The term "polyclonal" refers to antibodies that are heterogenous populations of antibody molecules derived from the sera of animals immunized with an antigen or an antigenic functional derivative thereof. For the production of polyclonal antibodies, various host animals may be immunized by injection with the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species.

"Monoclonal antibodies" are substantially homogenous populations of antibodies to a particular antigen. They may be obtained by any technique which provides for the production of antibody molecules by continuous cell lines in culture.

5 Monoclonal antibodies may be obtained by methods known to those skilled in the art. See, for example, Kohler, et al., *Nature* 256:495-497 (1975), and U.S. Patent No. 4,376,110.

10 The term "antibody fragment" refers to a portion of an antibody, often the hypervariable region and portions of the surrounding heavy and light chains, that displays specific binding affinity for a particular molecule. A hypervariable region is a portion of an antibody that physically binds to the polypeptide target.

15 Antibodies or antibody fragments having specific binding affinity to a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide may be used in methods for detecting the presence and/or amount of a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide in a sample by probing the sample with the antibody under conditions suitable for formation of an
20 immunocomplex between the antibody and the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide and detecting the presence and/or amount of the antibody conjugated to the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide. Diagnostic kits for performing such methods may be constructed to include
25 antibodies or antibody fragments specific for PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 as well as a conjugate of a binding partner of the antibodies or the antibodies themselves.

30 An antibody or antibody fragment with specific binding affinity to a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide can be isolated, enriched, or purified from a prokaryotic or eukaryotic organism. Routine methods known to those skilled in the art enable production of antibodies or antibody fragments, in both prokaryotic and eukaryotic

organisms. Purification, enrichment, and isolation of antibodies, which are polypeptide molecules, are described above.

In another aspect the invention features a hybridoma which
5 produces an antibody having specific binding affinity to a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide. By "hybridoma" is meant an immortalized cell line which is capable of secreting an antibody, for example a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 antibody. In
10 preferred embodiments the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 antibody comprises a sequence of amino acids that is able to specifically bind a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide.

In another embodiment, the invention encompasses a
15 recombinant cell or tissue containing a purified nucleic acid coding for a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide. In such cells, the nucleic acid may be under the control of its genomic regulatory elements, or may be under the control of exogenous regulatory elements including an
20 exogenous promoter. By "exogenous" it is meant a promoter that is not normally coupled transcriptionally to the coding sequence for the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide in its native state.

The invention features a method for identifying human
25 cells containing a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide or a related sequence. The method involves identifying the novel polypeptide in human cells using techniques that are routine and standard in the art, such as those described herein for identifying PTP04, SAD, PTP05,
30 PTP10, ALP, or ALK-7 (e.g., cloning, Southern or Northern blot analysis, in situ hybridization, PCR amplification, etc.).

The invention also features methods of screening cells for natural binding partners of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptides.

5 The term "natural binding partner" refers to molecules, or portions of these molecules, that bind to the protein of interest in cells. Natural binding partners may be polypeptides or lipids, but do not include glutathione. Natural binding partners can play a role in propagating a signal in a protein signal transduction process. A change in
10 the interaction between a protein and a natural binding partner can manifest itself as an increased or decreased probability that the interaction forms, or an increased or decreased concentration of the protein/natural binding partner complex.

A protein's natural binding partner can bind to a protein's
15 intracellular region with high affinity. High affinity represents an equilibrium binding constant on the order of 10^{-6} M or less. In addition, a natural binding partner can also transiently interact with a protein's intracellular region and chemically modify it. Natural binding partners of protein are
20 chosen from a group that includes, but is not limited to, SRC homology 2 (SH2) or 3 (SH3) domains, other phosphoryl tyrosine binding (PTB) domains, guanine nucleotide exchange factors, protein phosphatases, and other protein kinases or protein phosphatases. Methods of determining changes in interactions
25 between proteins and their natural binding partners are readily available in the art.

In another aspect, the invention provides an assay to identify substances capable of modulating the activity of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7. Such assays may be
30 performed in vitro or in vivo can be obtained by modifying existing assays, such as the assays described in WO 96/40276, published December 19, 1996 and WO 96/14433, published May 17, 1996. Other possibilities include testing for phosphatase

activity on standard substrates such as Src kinase or synthetic amino acid substrates. The substances so identified may be enhances or inhibitors of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity and can be peptides, natural products (such as those isolated from fungal strains, for example) or small molecular weight chemical compounds. A preferred substance will be a compound with a molecular weight of less than 5,000, more preferably less than 1,000, most preferably less than 500. The assay and substances contemplated by the invention are discussed in more detail below.

In a preferred embodiment, the invention provides a method for treating or preventing an abnormal condition by administering a compound which is a modulator of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 function *in vitro*. The abnormal condition preferably involves abnormality in PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 signal transduction pathway, and most preferably is cancer. Such compounds preferably show positive results in one or more *in vitro* assays for an activity corresponding to treatment of the disease or disorder in question (such as the assays described in examples 5, 10, 15, 20, and 21 below). Examples of substances that can be screened for favorable activity are provided in section XIV below.

Substances identified as modulators of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity can be used to study the effects of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 modulation in animal models of cell proliferative disorders. For example, inhibitors of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity can be tested as treatments for cell proliferative disorders such as leukemia or lymphoma using subcutaneous xenograph models in mice.

In a further aspect, the invention provides a method for identifying modulators of protein activity. The method involves the steps of: a) forming a captured protein by

contacting the protein with a natural binding partner; b) contacting the captured protein with a test compound; and c) measuring the protein activity. Preferably, the method also includes the step of comparing the protein activity with the activity of a control protein, which has the same amino acid sequence as the protein in step (a) without the natural binding partner, to determine the extent of modulation.

The term "modulator" refers to a compound which has the ability of altering the activity of a protein. A modulator may activate the activity of the protein, may activate or inhibit the activity of the protein depending on the concentration of the compound exposed to the protein, or may inhibit the activity of the protein.

The term "modulator" also refers to a compound that alters the function of a protein by increasing or decreasing the probability that a complex forms between a protein and a natural binding partner. A modulator preferably increases the probability that such a complex forms between the protein and the natural binding partner, more preferably increases or decreases the probability that a complex forms between the protein and the natural binding partner depending on the concentration of the compound exposed to the protein, and most preferably decreases the probability that a complex forms between the protein and the natural binding partner.

The term "activity of a protein", in the context of the invention, defines the natural function of a protein in a cell. Examples of protein function include, but are not limited to, catalytic activity and binding a natural binding partner.

The term "activates" refers to increasing the natural function of a protein. The protein function is preferably the interaction with a natural binding partner and most preferably catalytic activity.

The term "inhibit" refers to decreasing the cellular function of a protein. The protein function is preferably the interaction with a natural binding partner and most preferably catalytic activity.

5 The term "catalytic activity", in the context of the invention, defines the rate at which a protein reacts with a substrate. Catalytic activity can be measured, for example, by determining the amount of a substrate converted to a product as a function of time. When the protein is a protein kinase or a
10 protein phosphatase, then the reaction with a substrate is the phosphorylation or dephosphorylation of the substrate, respectively. Phosphorylation or dephosphorylation of a substrate occurs at the active-site of a protein kinase or a protein phosphatase. The active-site is normally a cavity in which the
15 substrate binds to the protein kinase or protein phosphatase and is phosphorylated.

 The term "substrate" as used herein refers to a molecule which is acted upon by an enzyme. If the enzyme is a protein kinase then the substrate is phosphorylated by the protein
20 kinase. If the enzyme is a protein phosphatase then the substrate is dephosphorylated by the protein phosphatase.

 The term "compound" refers to a molecule which has at least two types of atoms in its composition. The molecule may be a small organic molecule. The term "organic molecule" refers to a
25 molecule which has at least one carbon atom in its structure.

 The term "complex" refers to an assembly of at least two molecules bound to one another. Signal transduction complexes often contain at least two protein molecules bound to one another. For instance, a protein tyrosine receptor protein
30 kinase, GRB2, SOS, RAF, and RAS assemble to form a signal transduction complex in response to a mitogenic ligand.

 The term "contacting" as used herein refers to any touching between a compound and a protein, preferably the mixing of a

solution comprising a compound with a liquid medium bathing the protein of the methods. The touching may involve interaction between the compound and the protein. The solution comprising the compound may be added to the medium bathing the protein by
5 utilizing a delivery apparatus, such as a pipet-based device or syringe-based device.

The term "protein" as used herein refers to a naturally occurring or chemically modified polypeptide chain that has distinct secondary and tertiary structures. The chemical
10 modification may be point mutations. The term "protein" as used herein does not include a polypeptide chain which is covalently fused or otherwise joined through human intervention with another distinct polypeptide chain. For example, a GST-fusion protein is not included under the term "protein" as used herein.

15 The term "captured protein" as used herein refers to a protein that has come to contact with one of its natural binding partners and has formed a complex with the natural binding partner. The natural binding partner may be free in the solution, bound to a solid support, or free in the solution
20 with the ability to bind to a solid support.

The term "test compound" refers to a compound under study for its potential effect on the catalytic activity of a protein.

25 The term "control protein" refers to a protein which has the same amino acid sequence of the captured protein but is not being modulated by a test compound, nor has it come in contact with a test compound, nor is it bound to a natural binding partner. The activity of a control protein can be measured using the techniques of the invention, and such activity may be
30 compared with the activity of a modulated protein. A difference between the levels of the two measured activities determines the extent of modulation by the modulators.

The invention provides a method for identifying modulators of protein activity, where the method is preferably a non-radioactive method. The protein is preferably not a fusion protein. Most preferably, the protein is not a GST-fusion protein. The protein is preferably an enzyme, a receptor enzyme, or a non-receptor enzyme, more preferably a protein kinase, and most preferably a protein tyrosine kinase. The protein tyrosine kinase is preferably Zap70 or Syk. In other preferred embodiments, the protein is a protein tyrosine phosphatase, and more preferably the protein is PTP04, SAD, PTP05, PTP10, ALP, or ALK-7.

The term "fusion protein" refers to a heterologous protein formed by the covalent linkage of two distinct polypeptides. The term "GST-fusion protein" refers to a heterologous protein formed by the covalent linkage of a polypeptide and glutathione S-transferase (GST).

The term "enzyme" refers to a protein that can act as a catalyst for biological reactions. Examples of catalyzed biological reactions include, but are not limited to, formation of new bonds, addition of water, addition of a phosphoryl group, and isomerization of an organic molecule.

The term "catalyst" refers to a compound or a dissolved metal ion that increases the rate of a chemical reaction without being consumed in the reaction.

The term "receptor enzyme" refers to an enzyme that has a portion of its amino acid sequence within the cell membrane.

The term "non-receptor enzyme" refers to an enzyme that has none of its amino acid sequence within the cell membrane. The non-receptor enzyme may be associated with the membrane via interactions, such as covalent linkage with fatty acids of the membrane.

The term "protein kinase" refers to an enzyme that transfers the high energy phosphate of adenosine triphosphate

to an amino acid residue, either tyrosine, serine, or threonine, located on a protein target.

The term "protein tyrosine kinase," or PTK, refers to an enzyme that transfers the high energy phosphate of adenosine triphosphate to a tyrosine residue located on a protein target.

"Zap70" and "Syk" are protein tyrosine kinases of the Syk family which is characterized by the presence of two tandemly arranged Src-homology 2 (SH2) domains and no membrane localization motifs. These proteins are probably phosphorylated by the Src family of protein tyrosine kinases at the two tyrosine residues within the ITAM motif.

The term "ITAM motif" stands for "immunoreceptor tyrosine-based activation motif" and refers to a 16 amino acid motif (YXXLX₆₋₈YXXL) that is conserved in all of the signal transducing subunits of the T-cell antigen receptor (TCR) (*c.f.* Chan, et al. (1995) *The EMBO Journal*, 14:11, 2499-2508).

The term "protein tyrosine phosphatase" refers to an enzyme that removes a phosphate group from a phosphotyrosine in a protein target.

In a preferred embodiment, the natural binding partner of one of the above proteins is capable of binding to a solid support. The natural binding partner is preferably a peptide, more preferably a phosphopeptide, and most preferably the phosphopeptide comprises an ITAM motif. In other preferred embodiments, the natural binding partner comprises a lipid.

The term "solid support" as used herein refers to an insoluble surface to which a molecule can be bound. Examples of solid supports include, but are not limited to, well plates (*i.e.* 96-well plates), glass beads, or resins (*i.e.* cellulose, agarose, polypropylene, polystyrene, etc.). Natural binding partners can be attached, through either covalent or non-covalent interactions, to the solid support prior to or after binding a protein. Examples of non-covalent interactions

include, but are not limited to, hydrogen bonds, electrostatic interactions, and hydrophobic interactions.

The term "peptide" refers to an arrangement of two or more amino acids, linked together through an amide bond between the carboxyl end of one amino acid and the amino end of another.

The term "phosphopeptide" refers to a peptide that has a phosphate group chemically attached to one of its amino acid residues.

The term "lipid" refers to a water-insoluble substance that can be extracted from cells by organic solvents of low polarity. Examples of lipids include, but are not limited to, glycerides, steroids, and terpenes.

The modulators of protein activity being identified by the methods of the invention preferably modulate the autocatalytic activity, catalytic activity, or binding of a second natural binding partner.

The activity of an enzyme is "autocatalytic activity" when the enzyme and its substrate are identical. Some receptor protein tyrosine kinases are capable of exhibiting autocatalytic activity.

In preferred embodiments, the invention provides a method for identifying modulators of protein activity, comprising the step of contacting the captured protein with one or more components of the group consisting of a substrate, a second natural binding partner, and an antibody. The method preferably further involves the step of lysing cells before forming the captured protein. Most preferably, the method involves the step of washing the solid support after capturing the protein and binding the protein:natural binding partner complex to the solid support and prior to measuring the protein activity.

In another aspect, the invention provides a kit for the identification of modulators of non-receptor enzyme activity

comprising: a) a natural binding partner; b) a solid support; and c) one or more components selected from the group consisting of a substrate, a second natural binding partner, and an antibody.

5 The natural binding partner in the above kit is preferably a peptide, more preferably a phosphopeptide. Even more preferably the phosphopeptide comprises an ITAM motif. In other preferred embodiment, the natural binding partner comprises a lipid.

10 The summary of the invention described above is non-limiting and other features and advantages of the invention will be apparent from the following detailed description, and from the claims.

15 Brief Description of the Figures

Figure 1 shows a comparison between the amino acid sequence of human PTP04 and the amino acid sequence of the protein to which it is most closely related, murine ZPEP. The relative homology between the two (approximately 70%) suggests
20 that the two proteins are members of the same PTP family but are not species orthologs.

Detailed Description of the Invention

The present invention relates to the isolation and
25 characterization of new proteins which we have called PTP04, SAD, PTP05, PTP10, ALP, and ALK-7, nucleotide sequences encoding PTP04, SAD, PTP05, PTP10, ALP, or ALK-7, various products and assay methods that can be used to identify compounds useful for the diagnosis and treatment of various
30 PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 related diseases and conditions, for example cancer. Polypeptides derived from PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 and nucleic acids encoding such polypeptides may be produced using well known and

standard synthesis techniques when given the sequences presented herein.

I. The Polypeptides of the Invention

5

A. PTP04

PTP04 is a tyrosine phosphatase with an apparent molecular weight of approximately 100 kDa. Primary sequence analysis shows that PTP04 is comprised of three domains: an N-terminal domain, a catalytic domain, and a C-terminal domain. The lack of a hydrophobic stretch of amino acids generally characterized as a transmembrane region indicates that PTP04 is a non-receptor tyrosine phosphatase.

The full-length PTP04 was originally isolated from a human leukemia cell line. Subsequent expression analysis of both normal tissues and cancer cell lines, shown in detail below, revealed that PTP04 is expressed in human thymus and has very low expression in other normal cells but is significantly overexpressed in a number of tumors, particularly in leukemias and lymphomas. This suggests that PTP04 plays an important role in the growth and persistence of these cancers.

B. SAD

SAD is a tyrosine kinase with an apparent molecular weight of approximately 55 kDa. Primary sequence analysis shows that SAD is comprised of four domains: a domain at the N-terminus that shows no homology to any known sequence (the unique domain), an SH3 domain, an SH2 domain and a catalytic domain. The lack of a hydrophobic stretch of amino acids generally characterized as a transmembrane region indicates that SAD is a non-receptor tyrosine kinase. A comparison of the amino acid sequences suggests that SAD is a member of the Frk family. Like some other members of this family, SAD lacks an N-terminal

myristylation site and a C-terminal regulatory tyrosine characteristic of Src family members. It is most closely related to the murine NR-TK Srm (Kohmura, et al, Mol. Cell. Bio. 14(10):6915, 1994) with approximately 85% sequence
5 homology in the catalytic domain. (Discussed in detail in the examples below.)

SAD was originally isolated from a human breast cancer cell line. Subsequent expression analysis of both normal tissues and cancer cell lines, shown in detail below, revealed
10 that SAD has very limited expression in normal cells but is significantly overexpressed in a number of tumors. This suggests that SAD plays an important role in the growth and persistence of these cancers.

15 C. PTP05 and PTP10

PTP05 is a tyrosine phosphatase with an apparent molecular weight of approximately 49 kDa. Two additional isoforms have been identified, one larger (approximately 54 kDa) and one smaller (approximately 47 kDa). Primary sequence analysis
20 shows that PTP05 is comprised of three domains: an N-terminal domain, a catalytic domain, and a C-terminal domain. The lack of a hydrophobic stretch of amino acids generally characterized as a transmembrane region indicates that PTP05 is a non-receptor tyrosine phosphatase. PTP10 is also a tyrosine
25 phosphatase with significant homology to PTP05. Together they define a new family of PTPs.

D. ALP

ALP is a tyrosine phosphatase with an apparent molecular
30 weight of approximately 160 - 200 kDa. Primary sequence analysis shows that ALP is comprised of three domains: a domain at the N-terminus that is rich in proline residues (30.6%) and contains several tyrosines that may be

phosphorylated, a catalytic domain, and a C-terminal domain containing region rich in prolines and serines (45.6%) that resembling a PEST motif (Rogers, et al, Science 234:364, 1986). These proline rich regions may be protein:protein interaction sites as SH3 domains have been shown to bind to proline rich regions (Morton and Campbell, Curr. Biol. 4:614, 1994; Ren, et al, Science 259:1157, 1993). The lack of a hydrophobic stretch of amino acids generally characterized as a transmembrane region indicates that ALP is a non-receptor tyrosine phosphatase.

The full-length ALP was originally isolated from a human brain cancer cell line. Subsequent expression analysis of both normal tissues and cancer cell lines, shown in detail below, revealed that ALP has low expression in normal cells but is significantly overexpressed in a number of tumors. This suggests that ALP plays an important role in the growth and persistence of these cancers.

E. ALK-7

ALK-7 is a type I receptor serine/threonine kinase (STK receptor). Proteins with some homology have been described in the rat (Ryden, et al. J. Biol. Chem. 271:30603, 1996; Tsuchida, et al. Molec. Cell. Neurosci. 7:467, 1996), however, unlike the rat proteins, the human ALK-7 is expressed in more restricted regions of the brain, notably hippocampus, hypothalamic nuclei, substantia nigra, an pituitary. This extremely restricted expression pattern strongly suggests a role for human ALK-7 in the growth and/or survival of neurons and its relevance in treatment of such diseases as Parkinson's, Huntington's disease and Alzheimer's.

The polypeptide and nucleotide sequences of the invention can be used, therefore, to identify modulators of cell growth

and survival which are useful in developing therapeutics for various cell proliferative disorders and conditions, and in particular cancers related to inappropriate PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity. Assays to identify compounds that act intracellularly to enhance or inhibit PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity can be developed by creating genetically engineered cell lines that express PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 nucleotide sequences, as is more fully discussed below.

II. Nucleic Acids Encoding the Polypeptides of the Invention.

A first aspect of the invention features nucleic acid sequences encoding a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide. Included within the scope of this invention are the functional equivalents of the herein-described isolated nucleic acid molecules. Functional equivalents or derivatives can be obtained in several ways. The degeneracy of the genetic code permits substitution of certain codons by other codons which specify the same amino acid and hence would give rise to the same protein. The nucleic acid sequence can vary substantially since, with the exception of methionine and tryptophan, the known amino acids can be coded for by more than one codon. Thus, portions or all of the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene could be synthesized to give a nucleic acid sequence significantly different from that shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8. The encoded amino acid sequence thereof would, however, be preserved.

In addition, the nucleic acid sequence may comprise a nucleotide sequence which results from the addition, deletion or substitution of at least one nucleotide to the 5'-end and/or the 3'-end of the nucleic acid formula shown in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID

NO:6, SEQ ID NO:7, or SEQ ID NO:8, or a derivative thereof. Any nucleotide or polynucleotide may be used in this regard, provided that its addition, deletion or substitution does not alter the amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16 which is encoded by the nucleotide sequence. For example, the present invention is intended to include any nucleic acid sequence resulting from the addition of ATG as an initiation codon at the 5'-end of the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 nucleic acid sequence or its functional derivative, or from the addition of TTA, TAG or TGA as a termination codon at the 3'-end of the inventive nucleotide sequence or its derivative. Moreover, the nucleic acid molecule of the present invention may, as necessary, have restriction endonuclease recognition sites added to its 5'-end and/or 3'-end.

Such functional alterations of a given nucleic acid sequence afford an opportunity to promote secretion and/or processing of heterologous proteins encoded by foreign nucleic acid sequences fused thereto. All variations of the nucleotide sequence of the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 genes and fragments thereof permitted by the genetic code are, therefore, included in this invention.

Further, it is possible to delete codons or to substitute one or more codons by codons other than degenerate codons to produce a structurally modified polypeptide, but one which has substantially the same utility or activity of the polypeptide produced by the unmodified nucleic acid molecule. As recognized in the art, the two polypeptides are functionally equivalent, as are the two nucleic acid molecules which give rise to their production, even though the differences between the nucleic acid molecules are not related to degeneracy of the genetic code.

Functional equivalents or derivatives of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 can also be obtained using nucleic acid molecules encoding one or more functional domains of the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide.

5 The catalytic domain of PTP04 functions as an enzymatic remover of phosphate molecules bound onto tyrosine amino acids and a nucleic acid sequence encoding the catalytic domain alone or linked to other heterologous nucleic acid sequences can be considered a functional derivative of PTP04. Other functional
10 domains of PTP04 include, but are not limited to, the proline-rich region within the N-terminal domain, and the C-terminal domain. Nucleic acid sequences encoding these domains are shown in SEQ ID NO:1 as follows: N-terminal domain 53-196; catalytic domain 197-934, C-terminal domain 935-2473.

15 The SH2 domain of SAD functions as a phosphorylated tyrosine binding domain and a nucleic acid sequence encoding the SH2 domain alone or linked to other heterologous nucleic acid sequences can be considered a functional derivative of SAD. Other functional domains of SAD include, but are not
20 limited to, the unique domain, the SH3 domain, and the catalytic domain. Nucleic acid sequences encoding these domains are shown in SEQ ID NO:2 as follows: N-terminal unique domain approximately 49-213; SH3 domain approximately 214-375; SH2 domain approximately 406-684; catalytic domain
25 approximately 736-1488.

The catalytic domain of PTP05 functions to remove phosphate molecules bound onto tyrosine residues and a nucleic acid sequence encoding the catalytic domain alone or linked to other heterologous nucleic acid sequences can be considered a
30 functional derivative of PTP05. Other functional domains of these proteins include, but are not limited to, the proline-rich region within the N-terminal domain, and the C-terminal domain. Nucleic acid sequences encoding these domains are

shown in SEQ ID NO:3 as follows: N-terminal domain approximately 199-759 ; catalytic domain approximately 760-1458, C-terminal domain approximately 1459-1476.

5 The N-terminal proline-rich domain of ALP functions as a SH3 binding domain and a nucleic acid sequence encoding the N-terminal proline-rich domain alone or linked to other heterologous nucleic acid sequences can be considered a functional derivative of ALP. Other functional domains of ALP include, but are not limited to, the proline-rich region within
10 the N-terminal proline-rich domain, the C-terminal proline/serine-rich domain, the proline/serine-rich region within the C-terminal proline/serine-rich domain, and the catalytic domain. Nucleic acid sequences encoding these domains are shown in SEQ ID NO:7 as follows: N-terminal domain
15 313-2883; proline-rich region 1369-2643 ; catalytic domain approximately 2884-3600, C-terminal proline/serine-rich domain 3601-4134, proline/serine-rich region 3613-4456.

The extracellular domain of ALK-7 functions as a ligand or co-receptor binding domain and a nucleic acid sequence encoding
20 the extracellular domain alone or linked to other heterologous nucleic acid sequences can be considered a functional derivative of ALK-7. Other functional domains of ALK-7 include, but are not limited to, the signal sequence, the transmembrane domain, the intracellular domain, and the catalytic domain. Nucleic
25 acid sequences encoding these domains are shown in SEQ ID NO:8 as follows: signal sequence 155-229; extracellular domain 155-493; transmembrane domain 494-568; intracellular domain 569-1633; catalytic domain approximately 731-1609. It should be noted that the signal sequence is cleaved from the
30 extracellular domain in the mature protein.

III. A Nucleic Acid Probe for the Detection of the Proteins of the Invention.

A nucleic acid probe of the present invention may be used to probe an appropriate chromosomal or cDNA library by usual hybridization methods to obtain another nucleic acid molecule of the present invention. A chromosomal DNA or cDNA library may be prepared from appropriate cells according to recognized methods in the art (e.g. "Molecular Cloning: A Laboratory Manual", second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989).

In the alternative, chemical synthesis is carried out in order to obtain nucleic acid probes having nucleotide sequences which correspond to N-terminal and C-terminal portions of the amino acid sequence of the polypeptide of interest. Thus, the synthesized nucleic acid probes may be used as primers in a polymerase chain reaction (PCR) carried out in accordance with recognized PCR techniques, essentially according to PCR Protocols, "A Guide to Methods and Applications", edited by Michael et al., Academic Press, 1990, utilizing the appropriate chromosomal or cDNA library to obtain the fragment of the present invention.

One skilled in the art can readily design such probes based on the sequence disclosed herein using methods of computer alignment and sequence analysis known in the art (e.g.. "Molecular Cloning: A Laboratory Manual", second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, 1989). The hybridization probes of the present invention can be labeled by standard labeling techniques such as with a radiolabel, enzyme label, fluorescent label, biotin-avidin label, chemiluminescence, and the like. After hybridization, the probes may be visualized using known methods.

The nucleic acid probes of the present invention include RNA as well as DNA probes and nucleic acids modified in the sugar, phosphate or even the base portion as long as the probe still retains the ability to specifically hybridize under conditions as disclosed herein. Such probes are generated using techniques known in the art. The nucleic acid probe may be immobilized on a solid support. Examples of such solid supports include, but are not limited to, plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins, such as polyacrylamide and latex beads, and nitrocellulose. Techniques for coupling nucleic acid probes to such solid supports are well known in the art.

The test samples suitable for nucleic acid probing methods of the present invention include, for example, cells or nucleic acid extracts of cells, or biological fluids. The sample used in the above-described methods will vary based on the assay format, the detection method and the nature of the tissues, cells or extracts to be assayed. Methods for preparing nucleic acid extracts of cells are well known in the art and can be readily adapted in order to obtain a sample which is compatible with the method utilized.

IV. A Probe Based Method And Kit For Detecting the Proteins of the Invention.

One method of detecting the presence of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 in a sample comprises (a) contacting the sample with the above-described nucleic acid probe, under conditions such that hybridization occurs, and (b) detecting the presence of the probe bound to the nucleic acid molecule. One skilled in the art would select the nucleic acid probe according to techniques known in the art as described above. Samples to be tested include but should not be limited to RNA samples of human tissue.

A kit for detecting the presence of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 in a sample comprises at least one container having disposed therein the above-described nucleic acid probe. The kit may further comprise other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound nucleic acid probe. Examples of detection reagents include, but are not limited to radiolabelled probes, enzymatically labeled probes (horseradish peroxidase, Alkaline phosphatase), and affinity labeled probes (biotin, avidin, or streptavidin).

In detail, a compartmentalized kit includes any kit in which reagents are contained in separate containers. Such containers include small glass containers, plastic containers or strips of plastic or paper. Such containers allow the efficient transfer of reagents from one compartment to another compartment such that the samples and reagents are not cross-contaminated and the agents or solutions of each container can be added in a quantitative fashion from one compartment to another. Such containers will include a container which will accept the test sample, a container which contains the probe or primers used in the assay, containers which contain wash reagents (such as phosphate buffered saline, Tris-buffers, and the like), and containers which contain the reagents used to detect the hybridized probe, bound antibody, amplified product, or the like. One skilled in the art will readily recognize that the nucleic acid probes described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art with or without a set of instructions concerning the use of such reagents in an assay.

V. DNA Constructs Comprising a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 Nucleic Acid Molecule and Cells Containing These Constructs.

The present invention also relates to a recombinant DNA molecule comprising, 5' to 3', a promoter effective to initiate transcription in a host cell and the above-described nucleic acid molecules. In addition, the present invention relates to a recombinant DNA molecule comprising a vector and a nucleic acid molecule described herein. The present invention also relates to a nucleic acid molecule comprising a transcriptional region functional in a cell, a sequence complimentary to an RNA sequence encoding an amino acid sequence corresponding to a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide or functional derivative, and a transcriptional termination region functional in said cell. The above-described molecules may be isolated and/or purified DNA molecules.

The present invention also relates to a cell or organism that contains a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 nucleic acid molecule as described herein and thereby is capable of expressing a peptide. The polypeptide may be purified from cells which have been altered to express the polypeptide. A cell is said to be "altered to express a desired polypeptide" when the cell, through genetic manipulation, is made to produce a protein which it normally does not produce or which the cell normally produces at lower levels. One skilled in the art can readily adapt procedures for introducing and expressing either genomic, cDNA, or synthetic sequences into either eukaryotic or prokaryotic cells.

A nucleic acid molecule, such as DNA, is said to be "capable of expressing" a polypeptide if it contains nucleotide sequences which contain transcriptional and translational regulatory information and such sequences are "operably linked" to nucleotide sequences which encode the polypeptide. An

operable linkage is a linkage in which the regulatory DNA sequences and the DNA sequence sought to be expressed are connected in such a way as to permit gene sequence expression. The precise nature of the regulatory regions needed for gene
5 sequence expression may vary from organism to organism, but will in general include a promoter region which, in prokaryotes, contains both the promoter (which directs the initiation of RNA transcription) as well as the DNA sequences which, when transcribed into RNA, will signal synthesis
10 initiation. Such regions will normally include those 5'-non-coding sequences involved with initiation of transcription and translation, such as the TATA box, capping sequence, CAAT sequence, and the like.

If desired, the non-coding region 3' to the sequence
15 encoding a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene may be obtained by the above-described cloning methods. This region may be retained for its transcriptional termination regulatory sequences, such as termination and polyadenylation. Thus, by retaining the 3'-region naturally contiguous to the DNA
20 sequence encoding a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene, the transcriptional termination signals may be provided. Where the transcriptional termination signals are not satisfactorily functional in the expression host cell, then a 3' region functional in the host cell may be substituted.

25 Two DNA sequences (such as a promoter region sequence and a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 sequence) are said to be operably linked if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the
30 promoter region sequence to direct the transcription of a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene sequence, or (3) interfere with the ability of the a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene sequence to be transcribed by the promoter

region sequence. Thus, a promoter region would be operably linked to a DNA sequence if the promoter were capable of effecting transcription of that DNA sequence. Thus, to express a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene, transcriptional and translational signals recognized by an appropriate host are necessary.

The present invention encompasses the expression of a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene (or a functional derivative thereof) in either prokaryotic or eukaryotic cells. Prokaryotic hosts are, generally, very efficient and convenient for the production of recombinant proteins and are, therefore, one type of preferred expression system for a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene. Prokaryotes most frequently are represented by various strains of *E. coli*. However, other microbial strains may also be used, including other bacterial strains.

In prokaryotic systems, plasmid vectors that contain replication sites and control sequences derived from a species compatible with the host may be used. Examples of suitable plasmid vectors may include pBR322, pUC118, pUC119 and the like; suitable phage or bacteriophage vectors may include λ gt10, λ gt11 and the like; and suitable virus vectors may include pMAM-neo, pKRC and the like. Preferably, the selected vector of the present invention has the capacity to replicate in the selected host cell.

Recognized prokaryotic hosts include bacteria such as *E. coli* and those from genera such as *Bacillus*, *Streptomyces*, *Pseudomonas*, *Salmonella*, *Serratia*, and the like. However, under such conditions, the polypeptide will not be glycosylated. The prokaryotic host must be compatible with the replicon and control sequences in the expression plasmid.

To express PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 (or a functional derivative thereof) in a prokaryotic cell, it is

necessary to operably link a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 sequence to a functional prokaryotic promoter. Such promoters may be either constitutive or, more preferably, regulatable (i.e., inducible or derepressible). Examples of constitutive promoters include the int promoter of bacteriophage λ , the bla promoter of the b-lactamase gene sequence of pBR322, and the CAT promoter of the chloramphenicol acetyl transferase gene sequence of pPR325, and the like. Examples of inducible prokaryotic promoters include the major right and left promoters of bacteriophage λ (P_R and P_L), the trp, recA, lacZ, lacI, and gal promoters of E. coli, the α -amylase (Ulmanen et al., J. Bacteriol. 162:176-182, 1985) and the sigma-28-specific promoters of B. subtilis (Gilman et al., Gene sequence 32:11-20(1984)), the promoters of the bacteriophages of Bacillus (Gryczan, In: The Molecular Biology of the Bacilli, Academic Press, Inc., NY (1982)), and Streptomyces promoters (Ward et al., Mol. Gen. Genet. 203:468-478, 1986). Prokaryotic promoters are reviewed by Glick (J. Ind. Microbiot. 1:277-282, 1987); Cenatiempo (Biochimie 68:505-516, 1986); and Gottesman (Ann. Rev. Genet. 18:415-442, 1984).

Proper expression in a prokaryotic cell also requires the presence of a ribosome binding site upstream of the gene sequence-encoding sequence. Such ribosome binding sites are disclosed, for example, by Gold et al. (Ann. Rev. Microbiol. 35:365-404, 1981). The selection of control sequences, expression vectors, transformation methods, and the like, are dependent on the type of host cell used to express the gene.

As used herein, "cell", "cell line", and "cell culture" may be used interchangeably and all such designations include progeny. Thus, the words "transformants" or "transformed cells" include the primary subject cell and cultures derived therefrom, without regard to the number of transfers. It is also understood that all progeny may not be precisely identical

in DNA content, due to deliberate or inadvertent mutations. However, as defined, mutant progeny have the same functionality as that of the originally transformed cell.

Host cells which may be used in the expression systems of the present invention are not strictly limited, provided that they are suitable for use in the expression of the PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 peptide of interest. Suitable hosts may often include eukaryotic cells. Preferred eukaryotic hosts include, for example, yeast, fungi, insect cells, mammalian cells either in vivo, or in tissue culture. Mammalian cells which may be useful as hosts include HeLa cells, cells of fibroblast origin such as VERO, 3T3 or CHO-K1, or cells of lymphoid origin (such as 32D cells) and their derivatives. Preferred mammalian host cells include SP2/0 and J558L, as well as neuroblastoma cell lines such as IMR 332 and PC12 which may provide better capacities for correct post-translational processing.

In addition, plant cells are also available as hosts, and control sequences compatible with plant cells are available, such as the cauliflower mosaic virus 35S and 19S, and nopaline synthase promoter and polyadenylation signal sequences. Another preferred host is an insect cell, for example the *Drosophila* larvae. Using insect cells as hosts, the *Drosophila* alcohol dehydrogenase promoter can be used. Rubin, Science 240:1453-1459, 1988). Alternatively, baculovirus vectors can be engineered to express large amounts of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 in insect cells (Jasny, Science 238:1653, 1987); Miller et al., In: Genetic Engineering (1986), Setlow, J.K., et al., eds., Plenum, Vol. 8, pp. 277-297).

Any of a series of yeast gene sequence expression systems can be utilized which incorporate promoter and termination elements from the actively expressed gene sequences coding for glycolytic enzymes are produced in large quantities when yeast

are grown in mediums rich in glucose. Known glycolytic gene sequences can also provide very efficient transcriptional control signals. Yeast provides substantial advantages in that it can also carry out post-translational peptide modifications.

5 A number of recombinant DNA strategies exist which utilize strong promoter sequences and high copy number of plasmids which can be utilized for production of the desired proteins in yeast. Yeast recognizes leader sequences on cloned mammalian gene sequence products and secretes peptides bearing leader
10 sequences (*i.e.*, pre-peptides). For a mammalian host, several possible vector systems are available for the expression of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7.

A particularly preferred yeast expression system is that utilizing *Schizosaccharomyces pombe*. This system is useful
15 for studying the activity of members of the Src family (Superti-Furga, et al, EMBO J. 12:2625, 1993) and other NR-TKs.

A wide variety of transcriptional and translational regulatory sequences may be employed, depending upon the nature of the host. The transcriptional and translational regulatory
20 signals may be derived from viral sources, such as adenovirus, bovine papilloma virus, cytomegalovirus, simian virus, or the like, where the regulatory signals are associated with a particular gene sequence which has a high level of expression. Alternatively, promoters from mammalian expression products,
25 such as actin, collagen, myosin, and the like, may be employed. Transcriptional initiation regulatory signals may be selected which allow for repression or activation, so that expression of the gene sequences can be modulated. Of interest are regulatory signals which are temperature-sensitive so that by
30 varying the temperature, expression can be repressed or initiated, or are subject to chemical (such as metabolite) regulation.

Expression of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 in eukaryotic hosts requires the use of eukaryotic regulatory regions. Such regions will, in general, include a promoter region sufficient to direct the initiation of RNA synthesis.

5 Preferred eukaryotic promoters include, for example, the promoter of the mouse metallothionein I gene sequence (Hamer et al., J. Mol. Appl. Gen. 1:273-288, 1982); the TK promoter of Herpes virus (McKnight, Cell 31:355-365, 1982); the SV40 early promoter (Benoist et al., Nature (London) 290:304-310, 1981);
10 the yeast gal4 gene sequence promoter (Johnston et al., Proc. Natl. Acad. Sci. (USA) 79:6971-6975, 1982); Silver et al., Proc. Natl. Acad. Sci. (USA) 81:5951-5955, 1984).

Translation of eukaryotic mRNA is initiated at the codon which encodes the first methionine. For this reason, it is
15 preferable to ensure that the linkage between a eukaryotic promoter and a DNA sequence which encodes PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 (or a functional derivative thereof) does not contain any intervening codons which are capable of encoding a methionine (i.e., AUG). The presence of such codons
20 results either in a formation of a fusion protein (if the AUG codon is in the same reading frame as a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 coding sequence) or a frame-shift mutation (if the AUG codon is not in the same reading frame as a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 coding sequence).

25 A PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 nucleic acid molecule and an operably linked promoter may be introduced into a recipient prokaryotic or eukaryotic cell either as a nonreplicating DNA (or RNA) molecule, which may either be a linear molecule or, more preferably, a closed covalent circular
30 molecule (a plasmid). Since such molecules are incapable of autonomous replication, the expression of the gene may occur through the transient expression of the introduced sequence. Alternatively, permanent or stable expression may occur through

the integration of the introduced DNA sequence into the host chromosome.

A vector may be employed which is capable of integrating the desired gene sequences into the host cell chromosome. Cells which have stably integrated the introduced DNA into their chromosomes can be selected by also introducing one or more markers which allow for selection of host cells which contain the expression vector. The marker may provide for prototrophy to an auxotrophic host, biocide resistance, e.g., antibiotics, or heavy metals, such as copper, or the like. The selectable marker gene sequence can either be directly linked to the DNA gene sequences to be expressed, or introduced into the same cell by co-transfection. Additional elements may also be needed for optimal synthesis of single chain binding protein mRNA. These elements may include splice signals, as well as transcription promoters, enhancers, and termination signals. cDNA expression vectors incorporating such elements include those described by Okayama, Mol. Cell. Bio. 3:280, 1983.

The introduced nucleic acid molecule can be incorporated into a plasmid or viral vector capable of autonomous replication in the recipient host. Any of a wide variety of vectors may be employed for this purpose. Factors of importance in selecting a particular plasmid or viral vector include: the ease with which recipient cells that contain the vector may be recognized and selected from those recipient cells which do not contain the vector; the number of copies of the vector which are desired in a particular host; and whether it is desirable to be able to "shuttle" the vector between host cells of different species.

Preferred prokaryotic vectors include plasmids such as those capable of replication in E. coli (such as, for example, pBR322, ColE1, pSC101, pACYC 184, pVX. Such plasmids are, for example, disclosed by Sambrook (cf. "Molecular Cloning: A

Laboratory Manual", second edition, edited by Sambrook, Fritsch, & Maniatis, Cold Spring Harbor Laboratory, (1989)). Bacillus plasmids include pC194, pC221, pT127, and the like. Such plasmids are disclosed by Gryczan (In: The Molecular Biology of the Bacilli, Academic Press, NY (1982), pp. 307-329). Suitable Streptomyces plasmids include p1J101 (Kendall et al., J. Bacteriol. 169:4177-4183, 1987), and streptomyces bacteriophages such as fC31 (Chater et al., In: Sixth International Symposium on Actinomycetales Biology, Akademiai Kaido, Budapest, Hungary (1986), pp. 45-54). Pseudomonas plasmids are reviewed by John et al. (Rev. Infect. Dis. 8:693-704, 1986), and Izaki (Jpn. J. Bacteriol. 33:729-742, 1978).

Preferred eukaryotic plasmids include, for example, BPV, vaccinia, SV40, 2-micron circle, and the like, or their derivatives. Such plasmids are well known in the art (Botstein et al., Miami Wntr. Symp. 19:265-274, 1982); Broach, In: "The Molecular Biology of the Yeast Saccharomyces: Life Cycle and Inheritance", Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, p. 445-470 (1981); Broach, Cell 28:203-204, 1982); Bollon et al., J. Clin. Hematol. Oncol. 10:39-48, 1980); Maniatis, In: Cell Biology: A Comprehensive Treatise, Vol. 3, Gene Sequence Expression, Academic Press, NY, pp. 563-608 (1980).

Once the vector or nucleic acid molecule containing the construct(s) has been prepared for expression, the DNA construct(s) may be introduced into an appropriate host cell by any of a variety of suitable means, i.e., transformation, transfection, conjugation, protoplast fusion, electroporation, particle gun technology, calcium phosphate-precipitation, direct microinjection, and the like. After the introduction of the vector, recipient cells are grown in a selective medium, which selects for the growth of vector-containing cells. Expression of the cloned gene molecule(s) results in the

production of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 or fragments or functional derivatives thereof. This can take place in the transformed cells as such, or following the induction of these cells to differentiate (for example, by administration of bromodeoxyuracil to neuroblastoma cells or the like). A variety of incubation conditions can be used to form the peptide of the present invention. The most preferred conditions are those which mimic physiological conditions.

10 VI. The Polypeptides of the Invention.

Also a feature of the invention are PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptides. A variety of methodologies known in the art can be utilized to obtain the polypeptides of the present invention. They may be purified from tissues or cells which naturally produce them. Alternatively, the above-described isolated nucleic acid sequences can be used to express a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 protein recombinantly.

Any eukaryotic organism can be used as a source for the polypeptide of the invention, as long as the source organism naturally contains such a polypeptide. As used herein, "source organism" refers to the original organism from which the amino acid sequence is derived, regardless of the organism the protein is expressed in and ultimately isolated from.

25 One skilled in the art can readily follow known methods for isolating proteins in order to obtain the peptide free of natural contaminants. These include, but are not limited to: size-exclusion chromatography, HPLC, ion-exchange chromatography, and immuno-affinity chromatography.

30 A PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 protein, like all proteins, is comprised of distinct functional units or domains. In eukaryotes, proteins sorted through the so-called vesicular pathway (bulk flow) usually have a signal sequence

(also called a leader peptide) in the N- terminus, which is cleaved off after the translocation through the ER (endoplasmic reticulum) membrane. Some N-terminal signal sequences are not cleaved off, remaining as transmembrane segments, but it does not mean these proteins are retained in the ER; they can be further sorted and included in vesicles.

SAD protein lacks a hydrophobic signal sequence and is classified as a non-receptor protein. Other motifs involved in targeting proteins to specific cellular locations include those selective for the mitochondrial matrix (Gavel and von Heijne, Prot Eng 4:33, 1990), the nucleus (Robbins, et al, Cell 64:615, 1991), peroxisomes, endoplasmic reticulum (Jackson, et al, EMBO J 9:3253, 1990), vesicular pathways (Bendiak, Biophys Res Comm 170:879, 1990), glycosyl-phosphatidylinositol (GPI) lipid anchors, and lysosomal organelles, and motifs that target proteins to lipid membranes such as myristylation (Towler, et al, Annu Rev Biochem 57:69, 1988) and farnesylation sites. The N-terminal 15 amino acids of the SAD protein conforms to the features which define a mitochondrial membrane protein with a bipartite structure of an N-terminal stretch of high arginine content involved in membrane targeting followed by the apolar sequence which signals translocation to the mitochondrial intermembrane space.

Non-receptor proteins generally function to transmit signals within the cell, either by providing sites for protein:protein interactions or by having some catalytic activity (contained within a catalytic domain), often both. Methods of predicting the existence of these various domains are well known in the art. Protein:protein interaction domains can be identified by comparison to other proteins. The SH2 domain, for example is a protein domain of about 100 amino acids first identified as a conserved sequence region between the proteins Src and Fps (Sadowski, et al, Mol. Cell. Bio.

6:4396, 1986). Similar sequences were later found in many other intracellular signal-transducing proteins. SH2 domains function as regulatory modules of intracellular signaling cascades by interacting with high affinity to phosphotyrosine-containing proteins in a sequence specific and strictly phosphorylation-dependent manner (Mayer and Baltimore, Trends Cell. Biol. 3:8, 1993). Kinase or phosphatase catalytic domains can be identified by comparison to other known catalytic domains with kinase or phosphatase activity. See, for example Hanks and Hunter, FASEB J. 9:576-595, 1995.

Receptor proteins also have, and are somewhat defined by, a hydrophobic transmembrane segment(s) which are thought to be Alpha-helices in membranes. Membrane proteins also integrate into the cell membrane in a specific manner with respect to the two sides (cytoplasmic/intracellular or exo-cytoplasmic/extracellular), which is referred to as membrane topology. Extracellular portions of integral membrane proteins often function as ligand binding domains whereas intracellular portions generally function to transmit signals within the cell, either by providing sites for protein:protein interactions or by having some catalytic activity (contained within a catalytic domain), often both. Methods of predicting the existence of these various domains are well known in the art. See, for example, D. J. McGeoch, Virus Research 3:271, 1985, or G. von Heijne, Nucl. Acids Res. 14:4683, 1986, for signal sequences, P. Klein, et al., Biochim. Biophys. Acta 815:468, 1985, for transmembrane domains, and S. J. Singer, Ann. Rev. Cell Biol. 6:247, 1990, or E. Hartmann, et al., Proc. Natl. Acad. Sci. USA, 86:5786, 1989, for prediction of membrane topology. Kinase catalytic domains can be identified by comparison to other known catalytic domains with kinase activity. See, for example, Hanks and Hunter, FASEB J. 9:576-595, 1995.

Primary sequence analysis of the PTP04 amino acid sequence (shown in SEQ ID NO:9) reveals that it does not contain a signal sequence or transmembrane domain and is, therefore, an intracellular protein. Comparison to known protein sequences
5 reveals that PTP04 is comprised of several unique domains. These include a 48 amino acid N-terminal domain (shown from amino acid number 1-48 of SEQ ID NO:9), a 245 amino acid catalytic domain (shown from amino acid number 49-294 of SEQ ID NO:9), and a 512 amino acid C-terminal domain (shown from amino
10 acid number 295-807 of SEQ ID NO:9).

Primary sequence analysis of the SAD amino acid sequence (shown in SEQ ID NO:10) reveals that it contains four distinct domains. These include an approximately 55 amino acid N-terminal unique domain (shown from amino acid number 1-55 of
15 SEQ ID NO:10), an approximately 54 amino acid SH3 domain (shown from amino acid number 56-109 of SEQ ID NO:10), an approximately 93 amino acid SH2 domain (shown from amino acid number 120-212 of SEQ ID NO:10), an approximately 251 amino acid catalytic domain (amino acid number 230-480 of SEQ ID
20 No:10), and a C-terminal tail of 8 amino acids (shown from amino acid 481-488 of SEQ ID NO:10).

Primary sequence analysis of the PTP05 amino acid sequence (shown in SEQ ID NO:11 with isoforms shown in SEQ ID NO:12 and SEQ ID NO:13) reveals that it and its isoforms do not contain a
25 signal sequence or transmembrane domain, and it is, therefore, an intracellular protein. Comparison to known protein sequences reveals that PTP05 is comprised of several unique domains. These include a 187 amino acid N-terminal domain (shown from amino acid number 1-187 of SEQ ID NO:11), a 242
30 amino acid catalytic domain (shown from amino acid number 188-420 of SEQ ID NO:11), and a 5 amino acid C-terminal domain (shown from amino acid number 421-426 of SEQ ID NO:11).

Two additional isoforms of PTP05 were also identified, a "long" form (SEQ ID NO:12) and a "C-trunc" form (SEQ ID NO:13). The "long" form has a 37 amino acid insertion in the N-terminal domain (aminoacids 44-80 of SEQ ID NO:12) which extends this domain to 224 amino acids. The catalytic domain extends from amino acid 225-457 of SEQ ID NO:12 and the C-terminal domain extends from amino acids 458-463 of SEQ ID NO:12. The "C-trunc" form results from a deletion of nucleotides 1415-1507 of SEQ ID NO:3, most likely due to alternative exon splicing. This deletion results in a replacement of the C-terminal 21 amino acids with a unique 7 amino acid sequence. This change eliminates a conserved C-terminal portion of the catalytic domain, which may affect enzymatic activity. The N-terminal domain of the "C-trunc" form extends from amino acid 1-87 of SEQ ID NO:13, the catalytic domain from amino acids 188-405 of SEQ ID NO:13 and the unique C-terminal domain from 406-412 of SEQ ID NO:13.

Primary sequence analysis of the ALP amino acid sequence (shown in SEQ ID NO:15) reveals that it does not contain a signal sequence or transmembrane domain and is, therefore, an intracellular protein. Comparison to known protein sequences reveals that ALP is comprised of several unique domains. These include a 857 amino acid N-terminal proline-rich domain (shown from amino acid number 1-857 of SEQ ID NO:15) within which is a proline-rich region (amino acid number 353-777 of SEQ ID NO:15), a 238 amino acid catalytic domain (shown from amino acid number 858-1096 of SEQ ID NO:15), and a 177 amino acid C-terminal proline/serine-rich domain (shown from amino acid number 1097-1274 of SEQ ID NO:15) within which is a proline/serine-rich region (amino acid number 1101-1214 of SEQ ID NO:15).

Primary sequence analysis for an ALK-7 amino acid sequence (shown in SEQ ID NO:16) reveals that it contains all the motifs

characteristic of a type I STK receptor. These include a 25 amino acid signal peptide (shown from amino acid number 1-25 of SEQ ID NO:16), an 88 amino acid cysteine-rich extracellular region (shown from amino acid number 26-113 of SEQ ID NO:16), a
5 single 25 amino acid transmembrane domain (shown from amino acid number 114-136 of SEQ ID NO:16), and a 355 amino acid cytoplasmic domain (shown from amino acid number 137-493 of SEQ ID NO:16), which includes a GS domain and a catalytic domain (amino acid number 193-485 of SEQ ID NO:16).

10 The extracellular domain conserves the 10 cysteines present in all type I STK receptors (ten Dijke, et al., Oncogene 8:2879, 1993; Bassinge, et al., Science 263:87, 1994; Massague, Trends Cell Biol. 4:172, 1994) and also contains 3
15 potential N-linked glycosylation sites. The divergent extracellular domain sequence of ALK-7 (28-30% identity to ALK-4 and ALK-5) suggests it may have a unique ligand/type II STK receptor specificity. A rat ALK-7-like protein has been found to bind TGFbeta and activin in a complex with the type II TGF beta receptor and ACTRII. However, these ligands are not
20 expressed in the same cell types as human ALK-7 suggesting alternative ligands. Candidate ALK-7-specific ligands include other TGFbetas such as TGFbeta 2, GDF-1, and homologues of GDNF, such as neuturin, which have been found to be expressed in neurons in a pattern similar to that of ALK-7.

25 The intracellular domain is somewhat more homologous to other ALK proteins, particularly in the catalytic domain which shows 83% identity to other type I STK receptors. The 40 amino acids immediately N-terminal of the transmembrane domain (the juxtamembrane domain) are, however, quite unique in comparison
30 with other ALKs.

These PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 domains have a variety of uses. An example of such a use is to make a polypeptide consisting of the PTP04, SAD, PTP05, PTP10, ALP, or

ALK-7 catalytic domain and a heterologous protein such as glutathione S-transferase (GST). Such a polypeptide can be used in a biochemical assay for PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 catalytic activity useful for studying PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 substrate specificity or for identifying substances that can modulate PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 catalytic activity. Alternatively, one skilled in the art could create a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide lacking at least one of the three major domains. Such a polypeptide, when expressed in a cell, is able to form complexes with the natural binding partner(s) of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 but unable to transmit any signal further downstream into the cell, i.e., it would be signaling incompetent and thus would be useful for studying the biological relevance of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity. (See, for example, Gishizky, et al, PNAS :10889, 1995).

VII. An Antibody Having Binding Affinity To the Polypeptides of the Invention And A Hybridoma Containing the Antibody.

The present invention also relates to an antibody having specific binding affinity to an PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide. The polypeptide may have the amino acid sequence set forth in SEQ ID NO:2, or a be fragment thereof, or at least 6 contiguous amino acids thereof. Such an antibody may be identified by comparing its binding affinity to a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide with its binding affinity to another polypeptide. Those which bind selectively to PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 would be chosen for use in methods requiring a distinction between PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 and other polypeptides. Such methods could include, but should not be limited to, the analysis of altered PTP04, SAD, PTP05, PTP10, ALP, or ALK-7

expression in tissue containing other polypeptides and assay systems using whole cells.

A PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 peptide of the present invention can be used to produce antibodies or hybridomas. One skilled in the art will recognize that if an antibody is desired, such a peptide would be generated as described herein and used as an immunogen. Preferred PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 peptides for this purpose as shown in Example 4 below. The antibodies of the present invention include monoclonal and polyclonal antibodies, as well fragments of these antibodies, and humanized forms. Humanized forms of the antibodies of the present invention may be generated using one of the procedures known in the art such as chimerization or CDR grafting. The present invention also relates to a hybridoma which produces the above-described monoclonal antibody, or binding fragment thereof. A hybridoma is an immortalized cell line which is capable of secreting a specific monoclonal antibody.

In general, techniques for preparing monoclonal antibodies and hybridomas are well known in the art (Campbell, "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands, 1984; St. Groth et al., J. Immunol. Methods 35:1-21, 1980). Any animal (mouse, rabbit, and the like) which is known to produce antibodies can be immunized with the selected polypeptide. Methods for immunization are well known in the art. Such methods include subcutaneous or intraperitoneal injection of the polypeptide. One skilled in the art will recognize that the amount of polypeptide used for immunization will vary based on the animal which is immunized, the antigenicity of the polypeptide and the site of injection.

The polypeptide may be modified or administered in an adjuvant in order to increase the peptide antigenicity.

Methods of increasing the antigenicity of a polypeptide are well known in the art. Such procedures include coupling the antigen with a heterologous protein (such as globulin or β -galactosidase) or through the inclusion of an adjuvant during immunization.

For monoclonal antibodies, spleen cells from the immunized animals are removed, fused with myeloma cells, such as SP2/0-Ag14 myeloma cells, and allowed to become monoclonal antibody producing hybridoma cells. Any one of a number of methods well known in the art can be used to identify the hybridoma cell which produces an antibody with the desired characteristics. These include screening the hybridomas with an ELISA assay, western blot analysis, or radioimmunoassay (Lutz, et al., Exp. Cell Res. 175:109-124, 1988). Hybridomas secreting the desired antibodies are cloned and the class and subclass is determined using procedures known in the art (Campbell, "Monoclonal Antibody Technology: Laboratory Techniques in Biochemistry and Molecular Biology", supra, 1984).

For polyclonal antibodies, antibody containing antisera is isolated from the immunized animal and is screened for the presence of antibodies with the desired specificity using one of the above-described procedures. The above-described antibodies may be detectably labeled. Antibodies can be detectably labeled through the use of radioisotopes, affinity labels (such as biotin, avidin, and the like), enzymatic labels (such as horse radish peroxidase, Alkaline phosphatase, and the like), fluorescent labels (such as FITC or rhodamine, and the like), paramagnetic atoms, and the like. Procedures for accomplishing such labeling are well-known in the art, for example, see (Stemberger, et al., J. Histochem. Cytochem. 18:315, 1970; Bayer, et al., Meth. Enzym. 62:308, 1979; Engval, et al., Immunot. 109:129, 1972; Goding, J. Immunol. Meth. 13:215, 1976). The labeled antibodies of the present invention can be

used for in vitro, in vivo, and in situ assays to identify cells or tissues which express a specific peptide.

The above-described antibodies may also be immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir et al., "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10, 1986; Jacoby et al., Meth. Enzym. 34, Academic Press, N.Y., 1974). The immobilized antibodies of the present invention can be used for in vitro, in vivo, and in situ assays as well as in immunochromotography.

Furthermore, one skilled in the art can readily adapt currently available procedures, as well as the techniques, methods and kits disclosed above with regard to antibodies, to generate peptides capable of binding to a specific peptide sequence in order to generate rationally designed antipeptide peptides, for example see Hurby et al., "Application of Synthetic Peptides: Antisense Peptides", In Synthetic Peptides, A User's Guide, W.H. Freeman, NY, pp. 289-307(1992), and Kaspczak et al., Biochemistry 28:9230-8(1989).

VIII. An Antibody Based Method And Kit For Detecting the Polypeptides of the Invention.

The present invention encompasses a method of detecting a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide in a sample, comprising: (a) contacting the sample with an above-described antibody, under conditions such that immunocomplexes form, and (b) detecting the presence of said antibody bound to the polypeptide. In detail, the methods comprise incubating a test sample with one or more of the antibodies of the present

invention and assaying whether the antibody binds to the test sample. Altered levels, either an increase or decrease, of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 in a sample as compared to normal levels may indicate disease.

5 Conditions for incubating an antibody with a test sample vary. Incubation conditions depend on the format employed in the assay, the detection methods employed, and the type and nature of the antibody used in the assay. One skilled in the art will recognize that any one of the commonly available
10 immunological assay formats (such as radioimmunoassays, enzyme-linked immunosorbent assays, diffusion based Ouchterlony, or rocket immunofluorescent assays) can readily be adapted to employ the antibodies of the present invention. Examples of such assays can be found in Chard, "An Introduction to
15 Radioimmunoassay and Related Techniques" Elsevier Science Publishers, Amsterdam, The Netherlands (1986); Bullock et al., "Techniques in Immunocytochemistry," Academic Press, Orlando, FL Vol. 1(1982), Vol. 2 (1983), Vol. 3 (1985); Tijssen, "Practice and Theory of Enzyme Immunoassays: Laboratory Techni-
20 ques in Biochemistry and Molecular Biology," Elsevier Science Publishers, Amsterdam, The Netherlands (1985).

The immunological assay test samples of the present invention include cells, protein or membrane extracts of cells, or biological fluids such as blood, serum, plasma, or urine.
25 The test sample used in the above-described method will vary based on the assay format, nature of the detection method and the tissues, cells or extracts used as the sample to be assayed. Methods for preparing protein extracts or membrane extracts of cells are well known in the art and can be readily
30 be adapted in order to obtain a sample which is capable with the system utilized.

A kit contains all the necessary reagents to carry out the previously described methods of detection. The kit may

comprise: (i) a first container containing an above-described antibody, and (ii) second container containing a conjugate comprising a binding partner of the antibody and a label. In another preferred embodiment, the kit further comprises one or more other containers comprising one or more of the following: wash reagents and reagents capable of detecting the presence of bound antibodies.

Examples of detection reagents include, but are not limited to, labeled secondary antibodies, or in the alternative, if the primary antibody is labeled, the chromophoric, enzymatic, or antibody binding reagents which are capable of reacting with the labeled antibody. The compartmentalized kit may be as described above for nucleic acid probe kits. One skilled in the art will readily recognize that the antibodies described in the present invention can readily be incorporated into one of the established kit formats which are well known in the art.

IX. Isolation of Natural Binding Partners of the Polypeptides of the Invention.

The present invention also relates to methods of detecting natural binding partners capable of binding to a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide. A natural binding partner of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 may be, for example, a substrate protein which is dephosphorylated as part of a signaling cascade. The binding partner(s) may be present within a complex mixture, for example, serum, body fluids, or cell extracts.

In general methods for identifying natural binding partners comprise incubating a substance with PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 and detecting the presence of a substance bound to PTP04, SAD, PTP05, PTP10, ALP, or ALK-7.

Preferred methods include the two-hybrid system of Fields and Song (supra) and co-immunoprecipitation.

X. Identification of and Uses for Substances Capable of
5 Modulating the Activity of the Polypeptides of the
Invention.

The present invention also relates to a method of detecting a substance capable of modulating PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity. Such substances can either
10 enhance activity (agonists) or inhibit activity (antagonists). Agonists and antagonists can be peptides, antibodies, products from natural sources such as fungal or plant extracts or small molecular weight organic compounds. In general, small molecular weight organic compounds are preferred. Examples of
15 classes of compounds that can be tested for PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 modulating activity are, for example but not limited to, thiazoles (see for example co-pending US applications 60/033,522, 08/660,900), and naphthopyrones (US patent number 5,602,171).

20 In general the method comprises incubating cells that produce PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 in the presence of a test substance and detecting changes in the level of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity or PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 binding partner activity. A change
25 in activity may be manifested by increased or decreased phosphorylation of a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide, increased or decreased phosphorylation of a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 substrate, or increased or decreased biological response in cells. A method for detecting
30 modulation of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity using the phosphorylation of an artificial substrate is shown in the examples below. Biological responses can include, for example, proliferation, differentiation, survival, or motility.

The substance thus identified would produce a change in activity indicative of the agonist or antagonist nature of the substance. Once the substance is identified it can be isolated using techniques well known in the art, if not already
5 available in a purified form.

The present invention also encompasses a method of agonizing (stimulating) or antagonizing PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 associated activity in a mammal comprising administering to said mammal an agonist or antagonist to PTP04,
10 SAD, PTP05, PTP10, ALP, or ALK-7 in an amount sufficient to effect said agonism or antagonism. Also encompassed in the present application is a method of treating diseases in a mammal with an agonist or antagonist of PTP04-, SAD-, PTP05-, PTP10-, ALP-, or ALK-7-related activity comprising
15 administering the agonist or antagonist to a mammal in an amount sufficient to agonize or antagonize PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 associated function(s). The particular compound can be administered to a patient either by itself or in a pharmaceutical composition where it is mixed with suitable
20 carriers or excipient(s). In treating a patient a therapeutically effective dose of the compound is administered. A therapeutically effective dose refers to that amount of the compound that results in amelioration of symptoms or a prolongation of survival in a patient.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals. For example, for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population).
25 The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Compounds which exhibit large therapeutic indices are preferred. The data obtained from these cell culture assays
30

and animal studies can be used in formulating a range of dosage for use in human. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. For example, a dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ as determined in cell culture (i.e., the concentration of the test compound which achieves a half-maximal disruption of the protein complex, or a half-maximal inhibition of the cellular level and/or activity of a complex component). Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by HPLC.

The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g. Fingl et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p1).

It should be noted that the attending physician would know how to and when to terminate, interrupt, or adjust administration due to toxicity, or to organ dysfunctions. Conversely, the attending physician would also know to adjust treatment to higher levels if the clinical response were not adequate (precluding toxicity). The magnitude of an administered dose in the management of the oncogenic disorder of interest will vary with the severity of the condition to be treated and to the route of administration. The severity of the condition may, for example, be evaluated, in part, by standard prognostic evaluation methods. Further, the dose and perhaps dose frequency, will also vary according to the age, body weight, and

response of the individual patient. A program comparable to that discussed above may be used in veterinary medicine.

Depending on the specific conditions being treated, such agents may be formulated and administered systemically or locally. Techniques for formulation and administration may be found in "Remington's Pharmaceutical Sciences," 1990, 18th ed., Mack Publishing Co., Easton, PA. Suitable routes may include oral, rectal, transdermal, vaginal, transmucosal, or intestinal administration; parenteral delivery, including intramuscular, subcutaneous, intramedullary injections, as well as intrathecal, direct intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections, just to name a few.

For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks's solution, Ringer's solution, or physiological saline buffer. For such transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compositions of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Agents intended to be administered intracellularly may be administered using techniques well known to those of ordinary skill in the art. For example, such agents may be encapsulated into liposomes, then administered as described above.

5 Liposomes are spherical lipid bilayers with aqueous interiors. All molecules present in an aqueous solution at the time of liposome formation are incorporated into the aqueous interior. The liposomal contents are both protected from the external microenvironment and, because liposomes fuse with cell
10 membranes, are efficiently delivered into the cell cytoplasm. Additionally, due to their hydrophobicity, small organic molecules may be directly administered intracellularly.

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active
15 ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

In addition to the active ingredients, these
20 pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form
25 of tablets, dragees, capsules, or solutions.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or
30 lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active com-

pounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

XI. Transgenic Animals.

Also contemplated by the invention are transgenic animals useful for the study of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity in complex in vivo systems. A variety of methods are available for the production of transgenic animals associated with this invention. DNA sequences encoding PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 can be injected into the pronucleus of a fertilized egg before fusion of the male and female pronuclei, or injected into the nucleus of an embryonic cell (e.g., the nucleus of a two-cell embryo) following the initiation of cell division (Brinster, et al., Proc. Nat. Acad. Sci. USA 82: 4438, 1985). Embryos can be infected with viruses, especially retroviruses, modified to carry inorganic-ion receptor nucleotide sequences of the invention.

Pluripotent stem cells derived from the inner cell mass of the embryo and stabilized in culture can be manipulated in culture to incorporate nucleotide sequences of the invention. A transgenic animal can be produced from such cells through implantation into a blastocyst that is implanted into a foster mother and allowed to come to term. Animals suitable for transgenic experiments can be obtained from standard commercial

sources such as Charles River (Wilmington, MA), Taconic (Germantown, NY), Harlan Sprague Dawley (Indianapolis, IN), etc.

5 The procedures for manipulation of the rodent embryo and for microinjection of DNA into the pronucleus of the zygote are well known to those of ordinary skill in the art (Hogan, et al., supra). Microinjection procedures for fish, amphibian eggs and birds are detailed in Houdebine and Chourrout, *Experientia* 47: 897-905, 1991). Other procedures for intro-
10 duction of DNA into tissues of animals are described in U.S. Patent No., 4,945,050 (Sandford et al., July 30, 1990).

By way of example only, to prepare a transgenic mouse, female mice are induced to superovulate. After being allowed to mate, the females are sacrificed by CO₂ asphyxiation or
15 cervical dislocation and embryos are recovered from excised oviducts. Surrounding cumulus cells are removed. Pronuclear embryos are then washed and stored until the time of injection. Randomly cycling adult female mice are paired with vasectomized males. Recipient females are mated at the same time as donor
20 females. Embryos then are transferred surgically. The procedure for generating transgenic rats is similar to that of mice. See Hammer, et al., *Cell* 63:1099-1112, 1990).

Methods for the culturing of embryonic stem (ES) cells and the subsequent production of transgenic animals by the
25 introduction of DNA into ES cells using methods such as electroporation, calcium phosphate/DNA precipitation and direct injection also are well known to those of ordinary skill in the art. See, for example, *Teratocarcinomas and Embryonic Stem Cells, A Practical Approach*, E.J. Robertson, ed., IRL Press, 30 1987).

In cases involving random gene integration, a clone containing the sequence(s) of the invention is co-transfected with a gene encoding resistance. Alternatively, the gene

encoding neomycin resistance is physically linked to the sequence(s) of the invention. Transfection and isolation of desired clones are carried out by any one of several methods well known to those of ordinary skill in the art (E.J. Robertson, *supra*).

DNA molecules introduced into ES cells can also be integrated into the chromosome through the process of homologous recombination. Capecchi, *Science* 244: 1288-1292 (1989). Methods for positive selection of the recombination event (*i.e.*, neo resistance) and dual positive-negative selection (*i.e.*, neo resistance and gancyclovir resistance) and the subsequent identification of the desired clones by PCR have been described by Capecchi, *supra* and Joyner et al., *Nature* 338: 153-156, 1989), the teachings of which are incorporated herein. The final phase of the procedure is to inject targeted ES cells into blastocysts and to transfer the blastocysts into pseudopregnant females. The resulting chimeric animals are bred and the offspring are analyzed by Southern blotting to identify individuals that carry the transgene. Procedures for the production of non-rodent mammals and other animals have been discussed by others. See Houdebine and Chourrout, *supra*; Pursel, et al., *Science* 244:1281-1288, 1989); and Simms, et al., *Bio/Technology* 6:179-183, 1988).

Thus, the invention provides transgenic, nonhuman mammals containing a transgene encoding a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide or a gene effecting the expression of a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide. Such transgenic nonhuman mammals are particularly useful as an *in vivo* test system for studying the effects of introducing a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide, regulating the expression of a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide (*i.e.*, through the introduction of additional genes, antisense nucleic acids, or ribozymes).

A "transgenic animal" is an animal having cells that contain DNA which has been artificially inserted into a cell, which DNA becomes part of the genome of the animal which develops from that cell. Preferred transgenic animals are primates, mice, rats, cows, pigs, horses, goats, sheep, dogs and cats. The transgenic DNA may encode for a human PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide. Native expression in an animal may be reduced by providing an amount of anti-sense RNA or DNA effective to reduce expression of the receptor.

XII. Gene Therapy.

PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 or its genetic sequences, both mutated and non-mutated, will also be useful in gene therapy (reviewed in Miller, Nature 357:455-460, (1992). Miller states that advances have resulted in practical approaches to human gene therapy that have demonstrated positive initial results. The basic science of gene therapy is described in Mulligan, Science 260:926-931, (1993).

In one preferred embodiment, an expression vector containing a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 coding sequence or a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 mutant coding sequence as described above is inserted into cells, the cells are grown in vitro and then infused in large numbers into patients. In another preferred embodiment, a DNA segment containing a promoter of choice (for example a strong promoter) is transferred into cells containing an endogenous PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 in such a manner that the promoter segment enhances expression of the endogenous PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene (for example, the promoter segment is transferred to the cell such that it becomes directly linked to the endogenous PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 gene).

The gene therapy may involve the use of an adenovirus containing PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 cDNA targeted to an appropriate cell type, systemic PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 increase by implantation of engineered cells, injection with PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 virus, or injection of naked PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 DNA into appropriate cells or tissues, for example neurons.

Expression vectors derived from viruses such as retroviruses, vaccinia virus, adenovirus, adeno-associated virus, herpes viruses, several RNA viruses, or bovine papilloma virus, may be used for delivery of nucleotide sequences (e.g., cDNA) encoding recombinant PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 protein into the targeted cell population (e.g., tumor cells or neurons). Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors containing coding sequences. See, for example, the techniques described in Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y. (1989), and in Ausubel et al., *Current Protocols in Molecular Biology*, Greene Publishing Associates and Wiley Interscience, N.Y. (1989). Alternatively, recombinant nucleic acid molecules encoding protein sequences can be used as naked DNA or in reconstituted system e.g., liposomes or other lipid systems for delivery to target cells (See e.g., Felgner et al., *Nature* 337:387-8, 1989). Several other methods for the direct transfer of plasmid DNA into cells exist for use in human gene therapy and involve targeting the DNA to receptors on cells by complexing the plasmid DNA to proteins. See, Miller, *supra*.

In its simplest form, gene transfer can be performed by simply injecting minute amounts of DNA into the nucleus of a cell, through a process of microinjection. (Capecchi MR, *Cell* 22:479-88, 1980). Once recombinant genes are introduced into a

cell, they can be recognized by the cells normal mechanisms for transcription and translation, and a gene product will be expressed. Other methods have also been attempted for introducing DNA into larger numbers of cells. These methods include: transfection, wherein DNA is precipitated with CaPO_4 and taken into cells by pinocytosis (Chen C. and Okayama H, Mol. Cell Biol. 7:2745-52, 1987); electroporation, wherein cells are exposed to large voltage pulses to introduce holes into the membrane (Chu G., et al., Nucleic Acids Res., 15:1311-26, 1987); lipofection/liposome fusion, wherein DNA is packaged into lipophilic vesicles which fuse with a target cell (Felgner PL., et al., Proc. Natl. Acad. Sci. USA. 84:7413-7, 1987)); and particle bombardment using DNA bound to small projectiles (Yang NS. et al., Proc. Natl. Acad. Sci. 87:9568-72, 1990). Another method for introducing DNA into cells is to couple the DNA to chemically modified proteins.

It has also been shown that adenovirus proteins are capable of destabilizing endosomes and enhancing the uptake of DNA into cells. The admixture of adenovirus to solutions containing DNA complexes, or the binding of DNA to polylysine covalently attached to adenovirus using protein crosslinking agents substantially improves the uptake and expression of the recombinant gene. Curiel DT et al., Am. J. Respir. Cell. Mol. Biol., 6:247-52, 1992).

As used herein "gene transfer" means the process of introducing a foreign nucleic acid molecule into a cell. Gene transfer is commonly performed to enable the expression of a particular product encoded by the gene. The product may include a protein, polypeptide, anti-sense DNA or RNA, or enzymatically active RNA. Gene transfer can be performed in cultured cells or by direct administration into animals. Generally gene transfer involves the process of nucleic acid contact with a target cell by non-specific or receptor mediated

interactions, uptake of nucleic acid into the cell through the membrane or by endocytosis, and release of nucleic acid into the cytoplasm from the plasma membrane or endosome. Expression may require, in addition, movement of the nucleic acid into the nucleus of the cell and binding to appropriate nuclear factors for transcription.

As used herein "gene therapy" is a form of gene transfer and is included within the definition of gene transfer as used herein and specifically refers to gene transfer to express a therapeutic product from a cell *in vivo* or *in vitro*. Gene transfer can be performed *ex vivo* on cells which are then transplanted into a patient, or can be performed by direct administration of the nucleic acid or nucleic acid-protein complex into the patient.

In another preferred embodiment, a vector having nucleic acid sequences encoding a PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 is provided in which the nucleic acid sequence is expressed only in specific tissue. Methods of achieving tissue-specific gene expression as set forth in International Publication No. WO 93/09236, filed November 3, 1992 and published May 13, 1993.

In all of the preceding vectors set forth above, a further aspect of the invention is that the nucleic acid sequence contained in the vector may include additions, deletions or modifications to some or all of the sequence of the nucleic acid, as defined above.

In another preferred embodiment, a method of gene replacement is set forth. "Gene replacement" as used herein means supplying a nucleic acid sequence which is capable of being expressed *in vivo* in an animal and thereby providing or augmenting the function of an endogenous gene which is missing or defective in the animal.

XIII. Compounds that Modulate the Function of PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 Proteins.

In an effort to discover novel treatments for diseases, biomedical researchers and chemists have designed, synthesized, and tested molecules that inhibit the function of protein kinases. Some small organic molecules form a class of compounds that modulate the function of protein kinases. Examples of molecules that have been reported to inhibit the function of protein kinases include, but are not limited to, bis monocyclic, bicyclic or heterocyclic aryl compounds (PCT WO 92/20642, published November 26, 1992 by Maguire et al.), vinylene-azaindole derivatives (PCT WO 94/14808, published July 7, 1994 by Ballinari et al.), 1-cyclopropyl-4-pyridyl-quinolones (U.S. Patent No. 5,330,992), styryl compounds (U.S. Patent No. 5,217,999), styryl-substituted pyridyl compounds (U.S. Patent No. 5,302,606), certain quinazoline derivatives (EP Application No. 0 566 266 A1), selenoindoles and selenides (PCT WO 94/03427, published February 17, 1994 by Denny et al.), tricyclic polyhydroxylic compounds (PCT WO 92/21660, published December 10, 1992 by Dow), and benzylphosphonic acid compounds (PCT WO 91/15495, published October 17, 1991 by Dow et al.). The compounds that can traverse cell membranes and are resistant to acid hydrolysis are potentially advantageous therapeutics as they can become highly bioavailable after being administered orally to patients. However, many of these protein kinase inhibitors only weakly inhibit the function of protein kinases. In addition, many inhibit a variety of protein kinases and will therefore cause multiple side-effects as therapeutics for diseases.

Some indolinone compounds, however, form classes of acid resistant and membrane permeable organic molecules. PCT WO 96/22976, published August 1, 1996 by Ballinari et al. describes hydrosoluble indolinone compounds that harbor

tetralin, naphthalene, quinoline, and indole substituents fused to the oxindole ring. These bicyclic substituents are in turn substituted with polar moieties including hydroxylated alkyl, phosphate, and ether moieties. International Patent Publication WO 96/22976, published August 1, 1996 by Ballinari et al. describe indolinone chemical libraries of indolinone compounds harboring other bicyclic moieties as well as monocyclic moieties fused to the oxindole ring. WO 96/22976, published August 1, 1996 by Ballinari et al. teach methods of indolinone synthesis, methods of testing the biological activity of indolinone compounds in cells, and inhibition patterns of indolinone derivatives.

Other examples of substances capable of modulating PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity include, but are not limited to, tyrphostins, quinazolines, quinoxolines, and quinolines.

The quinazolines, tyrphostins, quinolines, and quinoxolines referred to above include well known compounds such as those described in the literature. For example, representative publications describing quinazoline include Barker et al., EPO Publication No. 0 520 722 A1; Jones et al., U.S. Patent No. 4,447,608; Kabbe et al., U.S. Patent No. 4,757,072; Kaul and Vougioukas, U.S. Patent No. 5, 316,553; Kreighbaum and Comer, U.S. Patent No. 4,343,940; Pegg and Wardleworth, EPO Publication No. 0 562 734 A1; Barker et al., Proc. of Am. Assoc. for Cancer Research 32:327 (1991); Bertino, J.R., Cancer Research 3:293-304 (1979); Bertino, J.R., Cancer Research 9(2 part 1):293-304 (1979); Curtin et al., Br. J. Cancer 53:361-368 (1986); Fernandes et al., Cancer Research 43:1117-1123 (1983); Ferris et al. J. Org. Chem. 44(2):173-178; Fry et al., Science 265:1093-1095 (1994); Jackman et al., Cancer Research 51:5579-5586 (1981); Jones et al. J. Med. Chem. 29(6):1114-1118; Lee and Skibo, Biochemistry 26(23):7355-7362

- (1987); Lemus et al., J. Org. Chem. 54:3511-3518 (1989); Ley and Seng, Synthesis 1975:415-522 (1975); Maxwell et al., Magnetic Resonance in Medicine 17:189-196 (1991); Mini et al., Cancer Research 45:325-330 (1985); Phillips and Castle, J. Heterocyclic Chem. 17(19):1489-1596 (1980); Reece et al., Cancer Research 47(11):2996-2999 (1977); Sculier et al., Cancer Immunol. and Immunother. 23:A65 (1986); Sikora et al., Cancer Letters 23:289-295 (1984); Sikora et al., Analytical Biochem. 172:344-355 (1988).
- 10 Quinoxaline is described in Kaul and Vougioukas, U.S. Patent No. 5,316,553.
- Quinolines are described in Dolle et al., J. Med. Chem. 37:2627-2629 (1994); MaGuire, J. Med. Chem. 37:2129-2131 (1994); Burke et al., J. Med. Chem. 36:425-432 (1993); and
- 15 Burke et al. BioOrganic Med. Chem. Letters 2:1771-1774 (1992).
- Tyrphostins are described in Allen et al., Clin. Exp. Immunol. 91:141-156 (1993); Anafi et al., Blood 82:12:3524-3529 (1993); Baker et al., J. Cell Sci. 102:543-555 (1992); Bilder et al., Amer. Physiol. Soc. pp. 6363-6143:C721-C730 (1991);
- 20 Brunton et al., Proceedings of Amer. Assoc. Cancer Rsch. 33:558 (1992); Bryckaert et al., Experimental Cell Research 199:255-261 (1992); Dong et al., J. Leukocyte Biology 53:53-60 (1993); Dong et al., J. Immunol. 151(5):2717-2724 (1993); Gazit et al., J. Med. Chem. 32:2344-2352 (1989); Gazit et al., " J. Med.
- 25 Chem. 36:3556-3564 (1993); Kaur et al., Anti-Cancer Drugs 5:213-222 (1994); Kaur et al., King et al., Biochem. J. 275:413-418 (1991); Kuo et al., Cancer Letters 74:197-202 (1993); Levitzki, A., The FASEB J. 6:3275-3282 (1992); Lyall et al., J. Biol. Chem. 264:14503-14509 (1989); Peterson et al.,
- 30 The Prostate 22:335-345 (1993); Pillemer et al., Int. J. Cancer 50:80-85 (1992); Posner et al., Molecular Pharmacology 45:673-683 (1993); Rendu et al., Biol. Pharmacology 44(5):881-888 (1992); Sauro and Thomas, Life Sciences 53:371-376 (1993);

Sauro and Thomas, J. Pharm. and Experimental Therapeutics 267(3):119-1125 (1993); Wolbring et al., J. Biol. Chem. 269(36):22470-22472 (1994); and Yoneda et al., Cancer Research 51:4430-4435 (1991).

- 5 Other compounds that could be used as modulators include oxindolinones.

Examples

The examples below are non-limiting and are merely
10 representative of various aspects and features of the present invention. The examples below show the isolation and characterization of the novel proteins, protein expression in normal and tumor cells, generation of protein specific antibodies, and recombinant expression in mammalian and yeast
15 systems. Also shown are assays useful for identifying compounds that modulate protein activity.

Example 1: Isolation Of cDNA Clones Encoding PTP04

The example below describes the isolation and identi-
20 fication of a new PTP sequence from primary cancer tissues and the subsequent cloning of a full-length human PTP04. Also described are probes useful for the detection of PTP04 in cells or tissues.

Materials and Methods:

Poly A+ RNA was isolated from 30uM cryostat sections of frozen samples from primary human lung and colon carcinomas (Micro-FastTrack, InVitrogen, San Diego, CA). This RNA was used to generate single-stranded cDNA using the Superscript
30 Preamplification System (GIBCO BRL, Gaithersburg, MD.; Gerard, GF et al. (1989), FOCUS 11, 66) under conditions recommended by the manufacturer. A typical reaction used 10 μ g total RNA or 2 μ g poly(A) RNA with 1.5 μ g oligo(dT)₁₂₋₁₈ in a reaction volume of

60 μ L. The product was treated with RNaseH and diluted to 100 μ L with H₂O. For subsequent PCR amplification, 1-4 μ L of this ssDNA was used in each reaction.

Degenerate oligonucleotides were synthesized on an Applied Biosystems 394 DNA synthesizer using established phosphoramidite chemistry, precipitated with ethanol and used unpurified for PCR. The sequence of the degenerate oligonucleotide primers follows:

PTPDFW = 5'-GAYTTYTG GVRNATGRTNTGGGA- (sense) (SEQ ID NO:17) and

PTPHCSA = 5'-CGGCCSAYNCCNGCNSWRCARTG -3' (antisense) (SEQ ID NO:18).

These primers were derived from the peptide sequences DFWXMXW(E/D) (SEQ ID NO:19) (sense strand from PTP catalytic domain) and HCXAGXG (antisense strand from PTP catalytic domain) (SEQ ID NO:20), respectively. Degenerate nucleotide residue designations are: N = A, C, G, or T; R = A or G; and Y = C or T.

PCR reactions were performed using degenerate primers applied to the single-stranded cDNA listed above. The primers were added at a final concentration of 5 μ M each to a mixture containing 10 mM Tris·HCl (pH8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each deoxynucleoside triphosphate, 0.001% gelatin, 1.5 U AmpliTaq DNA Polymerase (Perkin-Elmer/Cetus), and 1-4 μ L cDNA. Following 3 min denaturation at 95 °C, the cycling conditions were 94 °C for 30 s, 50 °C for 1 min, and 72 °C for 1 min 45 s for 35 cycles. PCR fragments migrating between 350-400 bp were isolated from 2% agarose gels using the GeneClean Kit (Bio101), and T-A cloned into the pCRII vector (Invitrogen Corp. U.S.A.) according to the manufacturer's protocol.

Colonies were selected for mini plasmid DNA-preparations using Qiagen columns and the plasmid DNA was sequenced using

cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction products were run on an ABI Prism 377 DNA Sequencer, and analyzed using the BLAST alignment algorithm (Altschul, S.F. et al., J. Mol. Biol. 215:403-10). One novel clone novel clone (G77-4a-117), designated PTP04, was isolated from human HLT370 primary lung carcinoma sample.

To obtain full-length cDNA encoding the novel phosphatase, RACE (rapid amplification of cDNA ends) was performed with sense or anti-sense oligonucleotides derived from the original PCR fragments. Marathon-Ready cDNA (Clontech, Palo Alto, CA) made from human Molt-4 leukemia cells was used in the RACE reactions with the following primers:

RACE primers:

5'-CACCGTTCGAGTATTTTCAGATTGTGAAGAAG-TCC-3' (6595) (SEQ ID NO:21),

5'-GGACTTCTTCACAATCTGAAATACTCGAACGGTG-3' (6596) (SEQ ID NO:22),

5'-CCGTTATGTGAGGAAGAGCCACATTACAGGACC-3' (6599) (SEQ ID NO:23),

5'-GGTCCTGTAATGTGGCTCTTCCTCACATAACGG-3' (6600) (SEQ ID NO:24),

AP-1, and AP-2 (Clontech).

RT-PCR primers for PTP04:

5'-GGCATGCATGGAGTATGAAATGG-3' (6618) (SEQ ID NO:25),

5'-CGTACATCCCAGATGAGCTCAAGAATAGGG-3' (6632) (SEQ ID NO:26).

Isolated cDNA fragments encoding PTP04 were confirmed by DNA sequencing and subsequently used as probes for the screening of a human leukocyte cDNA library.

A human leukocyte cDNA library (lTriplEx, Clontech) and a Molt-4 leukemia cell library (lgt11, Clontech) were then screened to isolate full-length transcripts encoding PTP04. The 5' or 3'-RACE fragments were ³²P-labeled by random priming and used as hybridization probes at 2x10⁶ cpm/mL following standard techniques for library screening. Pre-hybridization (3 h) and hybridization (overnight) were conducted at 42 °C in 5X SSC, 5 X Denhart's solution, 2.5% dextran sulfate, 50 mM Na₂PO₄/NaHPO₄ [pH 7.0], 50% formamide with 100 mg/mL denatured salmon sperm DNA. Stringent washes were performed at 65 °C in 0.1X SSC and 0.1% SDS. Several overlapping clones were isolated and found to span the sequence of the PCR fragment (G77-4a-117). The final sequence was verified by sequencing of both strains using a cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction products were run on an ABI Prism 377 DNA Sequencer.

Results:

The 3,580 bp human PTP04 nucleotide sequence encodes a polypeptide of 807 amino acids. The PTP04 coding sequence is flanked by a 52 nucleotide 5'-untranslated region and a 1086 nucleotide 3'-untranslated region ending with a poly(A) tail. While there are no upstream in frame stop codons, the first ATG beginning at nucleotide position 53 conforms to the Kozak consensus for an initiating methionine. This predicted first 6 amino acids are identical to those of murine ZPEP (SwissProt: P29352, GeneBank: M90388), further supporting this is the true translational start site. One cDNA clone had an insert after nucleotide 30 in the 5'UTR, but otherwise had no sequence differences.

The 807 amino acid sequence shows no signal sequence or a transmembrane domain and PTP04 is, therefore, an intracellular protein. PTP04 has an N-terminal region from amino acids 1-48,

a catalytic domain from amino acids 49-294, and a C-terminal tail from amino acids 295-807. PTP04 is most related to murine ZPEP with an overall homology of 70%. ZPEP is a member of a subfamily of PTPs that includes PTP-PEST, HSC, BDP1 and PTP20, all of which are cytoplasmic PTPs with a single catalytic domain and a region rich in Pro, Glu, Ser and Thr residues (PEST domain). PTP04 also has a C-terminal PEST domain, from amino acids 495-807, where there are 57 serine residues (18%) and 35 proline residues (11%). A comparison of the amino acid sequences of PTP04 and ZPEP is shown in Figure 1.

The homology between PTP04 and ZPEP is concentrated in the N terminal and C-terminal ends of the proteins with significant divergence in the middle. The N-terminal region of PTP04, from amino acids 1-48, is 81% homologous to murine ZPEP. The catalytic domain of PTP04, from amino acids 49-294, is 89% homologous to murine ZPEP. The region of PTP04 from 294-600 is approximately 50% homologous to murine ZPEP. The C-terminal region of PTP04, from 680-817, is 80% homologous to murine ZPEP. The human SuPTP04 sequence defines a novel member of the PTP-PEST subfamily of PTPs.

Example 2: Expression Of PTP04

The example below shows the evaluation of PTP04 expression in normal human tissues and in cancer cell lines.

Materials and Methods:

Northern blots were prepared by running 20 μ g total RNA per lane isolated from 22 human adult normal tissues (thymus, lung, duodenum, colon, testis, brain, cerebellum, salivary gland, heart, liver, pancreas, kidney, spleen, stomach, uterus, prostate, skeletal muscle, placenta, mammary gland, bladder, lymph node, adipose tissue), 2 human fetal normal tissues (fetal liver, fetal brain), and 24 human tumor cell lines (

HOP-92, EKVX, NCI-H23, NCI-H226, NCI-H322M, NCI-H460, A549, HOP-62, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, IGROV1, SK-OV-3, SNB-19, SNB-75, U251, SF-268, SF-295, SF-539, CCRF-CEM, SR, DU-145, PC-3) (obtained from Nick Scudero, National Cancer
5 Institute, Developmental Therapeutics Program, Rockville, MD). The total RNA samples were run on a denaturing formaldehyde 1% agarose gel and transferred onto a nitrocellulose membrane (BioRad, CA). An additional human normal tissue Northern blot containing 2 μ g polyA+ mRNA per lane from 8 different human
10 cancer cell lines (NCI-H522, K-562, MOLT-4, HL-60, S3, Raji, SW480, G361) on a charge-modified nylon membrane (human cancer cell line blot #7757-1, Clontech, Palo Alto, CA) were also hybridized.

For the total RNA samples, nitrocellulose membranes were
15 hybridized with randomly primed [α - 32 P]dCTP-labeled probes synthesized from a 579 bp StuI-BstXI fragment of pCR2.1.mini298. Hybridization was performed overnight at 42°C in 4X SSPE, 2.5X Denhardt's solution, 50% formamide, 0.2 mg/mL denatured salmon sperm DNA, 0.1 mg/mL yeast tRNA (Boehringer
20 Mannheim, IN), 0.2% SDS, with 5×10^6 cpm/mL of [α - 32 P]dCTP labeled DNA probes on a Techne hybridizer HB-1. The blots were washed with 2X SSC, 0.1% SDS, at 65 °C for 20 min twice followed by in 0.5 X SSC, 0.1% SDS at 65 °C for 20 min. The blots were exposed to a phospho-imaging screen for 24 hours and scanned on
25 a Molecular Dynamics Phosphoimager SF.

A 351 bp EcoRI-HindIII fragment of G77-4a-117 was used to generate a probe for 2 μ g poly A+ mRNA samples on a Clontech nylon membrane. Hybridization was performed at 42 °C overnight in 5X SSC, 2% SDS, 10X Denhardt's solution, 50% formamide, 100
30 μ g/mL denatured salmon sperm DNA with $1-2 \times 10^6$ cpm/mL of [α - 32 P]dCTP -labeled DNA probes. The membrane was washed at room temperature in 2X SSC/0.05% SDS for 30 min and followed by at

50 °C in 0.2X SSC/0.1% SDS for 30 min, twice, and exposed for 48 hours on Kodak XAR-2 film.

RT-PCR Detection of novel PTPs -

5 Total RNA was isolated from various cell lines or fresh frozen tissues by centrifugation through a cesium chloride cushion. Twenty μ g of total RNA was reverse transcribed with random hexamers and Moloney murine leukemia virus reverse transcriptase (Super-ScriptII, GIBCO BRL, Gaithersburg, MD).
10 PCR was then used to amplify cDNA encoding SuPTP04. RT-PCR reactions lacking only the reverse transcriptase were performed as controls. PCR products were electrophoresed on 3% agarose gels, visualized by ethidium bromide staining and photographed on a UV light box. The intensity for a 270-bp fragment
15 specific to PTP04 were compared among different RNA samples.

Results:

 A single SuPTP04 mRNA transcript of approximately 4.5 kb was identified by Northern analysis, and found to be
20 exclusively in the Thymus. The rest of 23 human normal tissues (fetal brain, fetal liver, lung, duodenum, colon, testis, brain, cerebellum, salivary gland, heart, liver, pancreas, kidney, spleen, stomach, uterus, prostate, skeletal muscle, placenta, mammary gland, bladder, lymph node, adipose tissue)
25 were all negative. Six of the human tumor cell lines (CCRF-CEM, K-562, MOLT-4, HL-60, SR, Raji) were positive. The rest of 26 human tumor cell lines were negative. RT-PCR with gene specific primer-pairs showed that expression of the transcripts encoding SuPTP04 confirmed the results from Northern analysis
30 and also detected low levels in adipose, kidney, small intestine, hematopoietic tissues and various cell types (spleen, thymus, lymph node, bone marrow, peripheral leukocytes and lymphocytes.

The selective expression of PTP04 in cells of hematopoietic origin including normal human thymus and several leukemia cell lines suggests a potential involvement in immune regulation including T and B cell survival, differentiation or co-stimulation, and/or inflammatory, immunosuppressive or autoimmune disorders. Additionally, expression in adipose tissue suggests a possible role in metabolic disorders such as diabetes.

10 Example 3: Recombinant Expression Of PTP04

The following example illustrates the construction of vectors for expression of recombinant PTP04 and the creation of recombinant cell lines expressing PTP04.

15 Construction of Expression Vectors -

Expression constructs were generated by PCR-assisted mutagenesis in which the entire coding domains of PTP04 was tagged on its carboxy-terminal end with the hemophilus influenza hemagglutinin (HA) epitope YPYDVPDYAS (SEQ ID NO:55) (Pati, 1992). The construct was introduced into two mammalian expression vectors: pLXSN (Miller, A.D. & Rosman, G.J., Biotechniques 7, 980-988, 1989) for the generation of virus producing lines; and pRK5 for transient expression in mammalian.

25 Dominant negative (signaling incompetent) PTP04 constructs were also made in both pLXSN and pRK5 by mutation of the invariant Cys in the conserved HCSAG (SEQ ID NO:56) motif to an Ala by PCR mutagenesis.

30 The entire PTP04 open reading frames (no HA-tag) excluding the initiating methionines were generated by PCR and ligated into pGEX vector (Pharmacia Biotech, Uppsala, Sweden) for bacterial production of GST-fusion proteins for immunization of rabbits for antibody production. The entire PTP04 open reading

frame excluding the initiating methionines was generated by PCR and ligated into pGEX vector for bacterial production of GST-fusion proteins for immunization of rabbits for antibody production. This vector contains the glutathione-S-transferase coding sequence followed by a polylinker for generating recombinant fusion proteins. The GST moiety comprises the N-terminal portion of the fusion protein.

Transient Expression in Mammalian Cells -

The pRK5 expression plasmids (10 µg DNA/100 mm plate) containing the HA-tagged PTP04 gene can be introduced into COS and 293 cells with lipofectamine (Gibco BRL). After 72 hours, the cells were harvested in 0.5 mL solubilization buffer (20 mM HEPES pH 7.35, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 1 µg/mL aprotinin). Sample aliquots were resolved by SDS polyacrylamide gel electrophoresis (PAGE) on 15%acrylamide/0.5% bis-acrylamide gels and electrophoretically transferred to nitrocellulose. Non-specific binding was blocked by preincubating blots in Blotto (phosphate buffered saline containing 5% w/v non-fat dried milk and 0.2% v/v nonidet P-40 (Sigma)), and recombinant protein was detected using a murine Mab to the HA decapeptide tag. Alternatively, recombinant protein can be detected using various PTP04-specific antisera.

Generation of Virus Producing Cell Lines

pLXSN recombinant constructs containing the PTP04 gene were transfected into an amphotropic helper cell line PA317 using CaCl₂ mediated transfection. After selection on G418, the cells were plated on normal media without G418 (500 µg/mL). Supernatants from resistant cells were used to infect the ecotropic helper cell line GP+E86, and cells again selected on

G418. Resistant cells were again taken off G418, and the supernatants harvested every 8-12 hours and pooled as virus stock. Redemann et al., 1992, Mol. Cell. Biol. 12: 491-498. Viral stock titers were typically $\sim 10^6$ /mL.

5

Stable Expression in Mammalian Cells

NIH-3T3, and BALB/3T3 cells were grown in 100 mm plates with DMEM (Gibco) containing 10% fetal calf serum (FCS). The cells were superinfected with the PTP04 retrovirus by adding approximately 3 mL viral supernatant to 15 mL culture media for approximately 24 hours. Cells expressing the retroviral constructs were then selected by growth in DMEM/10% FCS supplemented with 500 μ g/mL G418.

15 Example 4: Generation of Anti-PTP04 Antibodies

PTP04-specific immunoreagents were raised in rabbits against a mixture of three KLH-conjugated synthetic peptides corresponding to unique sequences present in human PTP04. The peptides (see below) were conjugated at the C-terminal residue with KLH.

peptide 428A: SWPPSGTSSKMSLDDLPEKQDGTVPSSLLP (SEQ ID NO:27)

peptide 429A: YSLPYDSKHQIRNASNVKHHDSALGVYSY (SEQ ID NO:28)

peptide 430A: HTLQADSYSPNLPKSTTKAAKMMNQORTKC (SEQ ID NO:29)

Additional immunoreagents were generated by immunizing rabbits with the bacterially expressed entire coding region of PTP04 expressed as a GST-fusion protein. GST fusion proteins were produced in DH5-alpha E. coli bacteria as described in

Smith, et al Gene 67:31, 1988. Bacterial protein lysates were purified on glutathione-sepharose matrix as described in Smith, et al, supra.

5 Example 5: Assay for PTP04 Activity

Materials and methods:

Recombinant wild-type and dominant negative (signaling incompetent) PTP04 (see Example 3, supra) were purified from bacteria as GST-fusion proteins. Lysates were bound to a
10 glutathione-sepharose matrix and washed twice with 1X HNTG, followed by one wash with a buffer containing 100 mM 2-(N-morpholino)ethansulfonic acid (MES), pH 6.8, 150 mM NaCl, and 1 mM EDTA.

The assay for phosphatase activity was essentially done as
15 described by Pei et al.(1993) using p-nitrophenolphosphate (PNPP) as a generic PTP substrate. Briefly, after the last washing step, reactions were started by adding 50 mL Assay Buffer (100 mM MES pH 6.8, 150 mM NaCl, 10 mM DTT, 2 mM EDTA, and 50 mM PNPP) to the matrix bound proteins. Samples were
20 incubated for 20 min. at 23 °C. The reactions were terminated by mixing 40 µL of each sample with 960 µL 1 N NaOH, and the absorbance of p-nitrophenol was determined at 450 nm. To control for the presence of PTP04 in the precipitates, the precipitates were boiled in SDS sample buffer and analyzed by
25 SDS-PAGE. The presence of PTP04 was then detected by immunoblot analysis with anti-PTP04 antibodies.

Example 6: Isolation and Characterization of SAD

This example describes the isolation and characterization
30 of the non-receptor tyrosine kinase SAD. Initially we set out to identify novel members of the Src family, a group of nine related cytoplasmic tyrosine kinases which play key roles in several signal transduction pathways. Based on comparison of

all known tyrosine kinases, we designed a pair of degenerate oligonucleotide primers that specifically recognize Src family members plus three more distantly related proteins Srm, Brk, and MKK3 or Frk (the Srm/Brk/Frk group). The sequence FGE/DVW (SEQ ID NO:30) is located near the amino terminus of the kinase domain and is unique to Src family members and the Srm/Brk/Frk group. The sequence WTAPE (SEQ ID NO: 31) is located just amino terminal to the highly conserved DVWS motif of tyrosine kinases and is contained in the Src family and the Srm/Brk/Frk group as well as the Eph, Csk, Abl, and Fes families.

When we used the FGE/DVW and WTAPE primers in PCR amplification reactions with HME (human mammary epithelial) cell ssDNA as a template, we isolated multiple copies of known Src relatives as well as a novel DNA fragment (HME 1264) of 483 bp with homology to other kinases. The novel sequence was most similar to mouse Srm (GeneBank Accession #D26186) and the clone was designated human SAD.

A SAD probe was used to screen a cDNA library constructed from human breast cancer cell line mRNA to isolate two overlapping, independent clones spanning the kinase domain, but containing apparent introns and presumably arising from incompletely processed transcripts. The 5' end of the coding region was subsequently isolated by sequential RACE reactions from MCF7 RNA, and the entire coding region was re-isolated by PCR from HME cDNA.

Materials And Methods

Total RNA was isolated using the Guanidine Salts/Phenol extraction protocol of Chomczynski and Sacchi (P. Chomczynski and N. Sacchi, Anal. Biochem. 162, 156 (1987) from HME (human mammary epithelial) cells. This RNA was used as a template to generate single-stranded cDNAs using the Superscript Pre-amplification System for First Strand Synthesis kit purchased

from GibcoBRL (Life Technologies, U.S.A.; Gerard, GF et al, FOCUS 11:66, 1989) under conditions recommended by manufacturer. A typical reaction used 10 μ g total RNA or 2 μ g poly(A)⁺ RNA with 1.5 μ g oligo(dT)₁₂₋₁₈ in a reaction volume of 5 60 μ L. The product was treated with RNaseH and diluted to 100 μ L with H₂O. For subsequent PCR amplification, 1-4 μ L of these ssCDNAs were used in each reaction.

Oligonucleotides were synthesized on an Applied Biosystems 394 DNA synthesizer using established phosphoramidite chemistry and were used unpurified after precipitation with ethanol. The 10 degenerate oligonucleotide primers are:

FGE/DVW = 5'-GGNCARTTYGGNGANGTNTGG-3' (SEQ ID NO:30) (sense)
and

WTAPE = 5'-CAGNGCNGCYTCNGGNGCNGTCCA-3' (SEQ ID NO:31)
15 (antisense).

These primers were derived from the peptide sequences GQFG(E/D)VW (SEQ ID NO:32) (sense strand) and WTAPEALL (SEQ ID NO:33) (antisense strand), respectively. Degenerate nucleotide residue designations are: N = A, C, G, or T; R = A or G; and Y 20 = C or T. Using Src as a template, these primers produce a product of 480 bp.

A PCR reaction was performed using primers FGE/DVW and WTAPE applied to HME cell cDNA. The primers were added at a final concentration of 0.5 μ M each to a mixture containing 10 25 mM Tris.HCl (pH8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each deoxynucleoside triphosphate, 0.001% gelatin, and 1.5 U AmpliTaq DNA Polymerase (Perkin-Elmer/Cetus), and 1-4 μ L cDNA. Following 3 min denaturation at 94 °C, the cycling conditions were 94 °C for 30 sec, 37 °C for 1 min, a 2 min ramp to 72 °C, 30 and 72°C for 1min for the first 3 cycles, followed by 94 °C for 30 sec, 60°C for 1 min, and 72 °C for 1 min for 35 cycles. PCR fragments migrating at between 450-550 bp were isolated from 2%

agarose gels, phosphorylated and repaired by treatment with T4 polynucleotide kinase and Klenow fragment, and blunt-end cloned into the EcoRV site of the vector pBluescriptSK+ (Stratagene U.S.A.).

5 Plasmid DNAs were isolated from single colonies by DNA minipreparations using QIAGEN columns and were sequenced using cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction products were run on an ABI Prism 377 DNA Sequencer, and
10 analyzed using the BLAST alignment algorithm (Altschul, S.F. et al., J. Mol. Biol. 215:403-10, 1990). A novel clone (HME1264) was isolated by PCR with primers FGE/DVW and WTape on single-stranded cDNA from HME cells as a template. This clone was subsequently designated as a fragment of human SAD.

15 A lambda ZapII (Stratagene Cloning Systems, La Jolla, CA) cDNA library was constructed using mRNA from a pool of breast carcinoma cell lines as a template for first strand cDNA synthesis with both oligo-(dT) and random priming (library created by Clontech custom library synthesis department, Palo
20 Alto, CA). The cell lines used for the pool were MCF7, HBL100, MDA-MB231, MDA-MB175IIV, MDA-MB435, MDA-MB453, MDA-MB468, BT20, T47D and SKBR3, all of which are available from the ATCC. Phage were screened on nitrocellulose filters with the random primed ³²P-labeled insert from HME1264 at 2x10⁶ cpm/mL in
25 hybridization buffer containing 6xSSPE, 50% formamide, 2x Denhardt's reagent, 0.1% SDS, with 0.05 mg/mL denatured, fragmented salmon sperm DNA. After overnight hybridization at 42 °C, filters were washed in 1xSSC, 0.1% SDS at 65 °C. Two overlapping partial clones were isolated and sequenced through
30 the coding region using manual sequencing with T7 polymerase and oligonucleotide primers (Tabor and Richardson, Proc. Natl. Acad. Sci. U.S.A. 84: 4767-71, 1987). These isolates encompass the kinase domain of SAD and extend from within an apparent

intron 5' to the kinase domain and extend 3' to an in-frame termination codon, but are interrupted by four more apparent introns.

Two sequential 5' RACE (rapid amplification of cDNA ends) reactions (Frohman et al., Proc. Natl. Acad. Sci. U.S.A. 85: 8998, 1988) were subsequently used to isolate the 5' end of the coding region. Single strand cDNA was prepared as described above using the Superscript Pre-amplification System (GibcoBRL) using 6 μ g total RNA from MCF7 cells as a template and gene specific primers 5556 (5'-AGTGAGCTTCATGTTGGCT-3' (SEQ ID NO:34) for RACE 1 or 5848 (5'-GGTAGAGGCTGCCATCAG-3' (SEQ ID NO:35)) for RACE 2 to prime reverse transcription. Following treatment with RNase H, ssDNA was recovered using two sequential ethanol precipitations with ammonium acetate and carrier glycogen homopolymer tail of dA was added by treatment with deoxy-terminal transferase (GibcoBRL) and two reaction mixtures diluted to 50 μ L with TE. Second strand cDNA synthesis by AmpliTaq DNA Polymerase (Perkin-Elmer/Cetus) was primed with 0.2 μ M PENN(dT)₁₇ (5'- GACGATCGGAATTCGCGA(dT)₁₇-3' (SEQ ID NO:36) using 1-5 μ L of tailed cDNA as a template and buffer conditions given above. Following 5 min denaturation at 94 °C, 1 min annealing at 50 °C, and 40 min extension at 72 °C, primers PENN (5'-GACGATCGGAATTCGCGA-3' (SEQ ID NO:37) and 5555 (5'-CCCAGCCACAGGCCTTC-3' (SEQ ID NO:38) were added at 1 μ M and PCR done with cycling conditions of 94 °C for 30 s, 49 °C for 1 min, and 72 °C for 1 min, 45 sec for 40 cycles. A second, nested PCR was done using 0.2 μ L of the initial PCR reaction as a template and primers PENN (see SEQ ID NO:37) and 5554 (5'-CCACACCTCCCCAAAGTA-3' (SEQ ID NO:39) at 1 μ M with an initial 3 min denaturation at 94 °C, followed by cycling conditions of 94 °C for 30 s, 49 °C for 1 min, and 72 °C for 1 min, 45 sec for 35

cycles. PCR products were separated on 1% agarose gels and visualized by ethidium bromide staining and Southern hybridization using oligonucleotide 5557 (5'-TGGGAGCGGCCACACTCCGAATTCGCCCTT-3' (SEQ ID NO:40) end-labeled with ³²P. Reaction products of 500-700 bp were digested with EcoRI and cloned into the EcoRI site of pBluescriptSK+ (Stratagene U.S.A.), and positive clones were identified by colony hybridization with oligonucleotide 5557 as a probe. Clone 16A1 (which encompasses nucleotides 195 to 783 of SEQ ID NO:10) was isolated and sequenced by a combination of ABI and manual sequencing.

A second set of 5' RACE reactions was done based on the sequence of clone 16A1 using the procedure given above except as noted. Gene specific primers were 5848 (SEQ ID NO:35) for the first strand synthesis, 6118 (5'-GCCTGCGTGCGAAGATG-3' (SEQ ID NO:41) for the first PCR, and 6119 (5'-CTTCGAGGGCACAGAGCC-3' (SEQ ID NO:42) for the second PCR, and the probe for Southern and colony hybridization was random primed ³²P-labeled insert from 16A1. PCR fragments migrating at between 250-450 bp were isolated from 2% agarose gels, phosphorylated and repaired by treatment with T4 polynucleotide kinase and Klenow fragment, and blunt-end cloned into the EcoRV site of the vector pBluescriptSK+ (Stratagene U.S.A.). Clone 20E2 (which encompasses nucleotides 1 to 267 of SEQ ID NO:10) was isolated and sequenced by a combination of ABI and manual sequencing.

The coding region of SAD was subsequently isolated from HME cDNA as two overlapping PCR fragments. Single stranded cDNA was prepared from poly(A)+ RNA from HME cells using the Superscript Preamplification System (GibcoBRL) as described above. PCR with AmpliTaq DNA Polymerase (Perkin-Elmer/Cetus) used 1-2 μ L of cDNA as a template, an initial 3 min denaturation at 94°C, followed by cycling conditions of 94°C for

30 s, 55 °C for 1 min, and 72 °C for 1 min, 45 sec for 30 cycles and the buffer conditions given above. Primers 6642 (5'-ATGGAGCCGTTTCCTCAGGAGG-3' (SEQ ID NO:43) and 6644 (5'-TCACCCAGCTTCCTCCCAAGG-3' (SEQ ID NO:44) were used to amplify an approximately 710 bp 5' fragment of SAD, and primers 6643 (5'-AGGCCAACTGGAAGCTGATCC-3' (SEQ ID NO:45) and 6645 (5'-GCTGGAGCCCAGAGCGTTGG-3' (SEQ ID NO:46) were used to amplify an approximately 860 bp 3' fragment of SAD. PCR fragments were isolated from 1% agarose gels, phosphorylated and repaired by treatment with T4 polynucleotide kinase and Klenow fragment, and blunt-end cloned into the EcoRV site of the vector pBluescriptSK+ (Stratagene U.S.A.). Positive clones were identified by colony hybridization with the random primed 32P-labeled insert from 16A1 (for the 5' fragment) and the random primed 32P-labeled insert from HME1264 or 32P-labeled oligonucleotide 5557 (for the 3' fragment) as probes. The overlapping 5' and 3' PCR fragments were ligated together via the unique EcoRI site to give the full length SAD coding region. The complete sequence of the coding region of human SAD was determined from overlapping 5' and 3' PCR clones amplified from cDNA prepared from HME cells. 5' noncoding sequence was determined from the overlapping RACE fragment 16A1. Sequence was determined manually on both strands using cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA).

Results

The 1,548 bp human SAD (SAD_h) nucleotide sequence shown in SEQ ID NO:10 contains a single open reading frame encoding a polypeptide of 488 amino acids. The SAD_h coding region is preceded by a 48 nucleotide 5'-untranslated region including an in-frame termination codon four codons before the initiating

methionine and a 33 nucleotide 3'-untranslated region that includes two in-frame stop codons.

The sequences of SAD cDNAs were determined from overlapping PCR-amplified fragments from normal HME cell cDNA (nucleotides 49-1548), clones from a breast carcinoma cell lambda cDNA library (nucleotides 694-1548), and overlapping 5' RACE products from MCF7 cDNA (nucleotides 1-783) with the following sequence differences including some likely polymorphic sites. Ambiguities include a change of nucleotide 636 (see SEQ ID NO:10) from a C in the HME PCR clone to a T in the MCF7 RACE product, nucleotide 1477 from a T in the HME PCR clone to a C in the breast carcinoma library, a deletion of nucleotides GT at positions 919 - 920 in the breast carcinoma library and apparent introns inserted at positions (relative to SEQ ID NO:10) 694, 995, 1117, and 1334 in the breast carcinoma library.

The domain structure of SAD consists of an N-terminal unique domain followed by an SH3 domain, an SH2 domain and a kinase domain. This overall topology is shared by members of the Src, Srm/Brk/Mkk3, and Csk families. SAD is most similar to mouse Srm (GeneBank Accession #D26186) (Kohmura et al., Mol. Cell. Biol. 14: 6915-6925, 1994), a distant SRC relative of unknown function. SAD and Srm share sequence identities in the individual domains of 55% (unique region), 72% (SH3 domain), 78% (SH2 domain), and 85% (kinase domain). Unlike true Src family members, SAD and Srm lack both an N-terminal membrane attachment sequence and a potential C-terminal negative regulatory tyrosine. In addition, the characteristic HRDLRXAN (SEQ ID NO:47) sequence in the Src family kinase domain is HRDLAXRN (SEQ ID NO:48) in SAD and other Srm/Brk/Mkk3 group members. Like most other tyrosine kinases, SAD and Srm both contain a potential autophosphorylation site (380Y of SAD). The N-terminal sequences of SAD and Srm are similar with twenty

identical residues out of the first twenty-two amino acids, but the extreme C-termini are quite distinct.

Available evidence suggests that SAD_h and Srm_m are distinct genes rather than species orthologues. Overall, the levels of homology between SAD_h and Srm_m listed above are comparable to those of close Src family members (for example Src_h and Yes_h), but lower than those of species counterparts (for example Src_h and Src_m). SAD_h and Srm_m also exhibit distinct expression patterns with SAD_h expression detected by PCR in the duodenum and perhaps testes, but not in other tissues tested, while the Srm_m expression was detected by Northern with highest levels in lung, liver, spleen, kidney, and testes (Kohmura et al., Mol. Cell. Biol. 14: 6915-6925, 1994) (See Example 2 below.). Lastly, disruption of the Srm gene in mice has no detectable phenotype (Kohmura et al., Mol. Cell. Biol. 14: 6915), suggesting that other related proteins might compensate for its function.

Example 7: SAD Expression Analysis

Materials And Methods

RNA was isolated from a variety of human cell lines and fresh frozen normal tissues. (Tumor cell lines were obtained from Nick Scudero, National Cancer Institute, Developmental Therapeutics Program, Rockville, MD) Single stranded cDNA was synthesized from 10 µg of each RNA as described above using the Superscript Preamplification System (GibcoBRL). These single strand templates were then used in a 35 cycle PCR reaction using an annealing temperature of 65 °C with two SAD-specific oligonucleotides (5284: 5'-TCGCCAAGGAGATCCAGACAC-3' (SEQ ID NO:49), and 5285: 5'-GAAGTCAGCCACCTTGAGGC-3' (SEQ ID NO:50). Reaction products were electrophoresed on 2% agarose gels,

stained with ethidium bromide and photographed on a UV light box. The relative intensity of the approximately 320-bp SAD-specific band was estimated for each sample. The results are shown with a numerical rating with 4 being the highest relative expression and 0 being the lowest.

Results

The SAD expression profile in normal human tissue and multiple cell lines of diverse neoplastic origin was determined by the semi-quantitative PCR assay using primers from sequences in the kinase domain. The results are included in Tables 1 and 2. In normal tissue samples (Table 1), modest SAD expression was detected in the duodenum and possible low levels in testes with all other samples negative. Much higher expression was found in a subset of cancer cell lines (Table 2) with the highest levels in some human colon tumor cell lines (HCT-15, SW480, and HT-29), an ovarian carcinoma (IGROV1), and an intestinal carcinoma (SNU-C2B). Lesser expression of SAD was also seen in some other tumor cell lines derived from colon, breast, lung, ovary, and kidney as shown in Table 2.

Table 1

	cell type	Origin	exp. level
	duodenum	Normal Tissue	2
	testes	Normal Tissue	1?
5	brain	Normal Tissue	0
	heart	Normal Tissue	0
	kidney	Normal Tissue	0
	lung	Normal Tissue	0
	pancreas	Normal Tissue	0
10	placenta	Normal Tissue	0
	salivary gland	Normal Tissue	0
	skeletal muscle	Normal Tissue	0
	spleen	Normal Tissue	0
	stomach	Normal Tissue	0
15	thymus	Normal Tissue	0
	cerebellum	Normal Tissue	0
	liver	Normal Tissue	0
	uterus	Normal Tissue	0
	prostate	Normal Tissue	0

Table 2

	Cell Line	Origin	exp.	Cell Line	Origin	exp.
	HCT-15	colon	4	LOX IMVI	melanoma	1?
5	IGROV1	ovary	4	KATO III	gastric carcinoma	0
	SW480	colon adenocarcinoma	3	R-48	meta gast. adenocarcinoma	0
10	SNU-C2B	cecum primary carcinoma	3	HFL1	lung, diploid	0
	HT-29	colon	3	HOP62	lung	0
15	Colo 205	colon carcinoma	2	OVCAR-4	ovary	0
	SW948	colon adenocarcinoma	2	SKOV3	ovary	0
20	HCT116	colon	2	NCIH23	lung	0
	EKVX	lung	2	NCI-H460	lung	0
	NCI-H23	lung	2	COLO205	colon	0
	HCC-2998	colon	2	NCI-H460	lung	0
25	HCT116	colon	2	A549/ATCC	LUNG	0
	MCF7	breast	2	HOP-62	lung	0
	T-47D	breast	2	COLO 205	colon	0
	OVCAR-3	ovary	2	KM-12	colon	0
30	OVCAR-5	ovary	2	MDA-MB-231	breast	0
	OVCAR-8	ovary	2	MDA-MB-435	breast	0
	SN12C	renal	2	MDA-N	breast	0
	ACHN	renal	2	BT-549	breast	0
35	786-0	renal	2	SNB-19	CNS	0
	TK-10	renal	2	SNB-75	CNS	0
	HT29	colon adenocarcinoma	1	U251	CNS	0
40	RF-1	gastric carcinoma	1	SF-268	CNS	0
	AGS	gastric carcinoma	1	SF-295	CNS	0
45						

	EKVX	lung	1	CCRF-CEM	leukemia	0
	HOP-92	lung	1	MOLT-4	leukemia	0
	NCI-H226	lung	1	HL-60 (TB)	leukemia	0
	NCI-H322M	lung	1	RPMI8226	leukemia	0
5	MCF7/ADR	breast	1	SR	leukemia	0
	OVCAR-4	ovary	1	UO-31	renal	0
	SF-539	CNS	1	A498	renal	0
	K-562	leukemi a	1	Caki-1	renal	0
10	RXF393	renal	1	SK-MEL-2	melanoma	0
	Calu-3	lung adenoca rcinoma	1?	SK-MEL-5	melanoma	0
	NCI-H522	lung	1?	SK-MEL-28	melanoma	0
15	SW620	colon	1?	UACC-62	melanoma	0
	Hs578T	breast	1?	UACC-257	malanoma	0
	Sk-OV-3	ovary	1?	M14	melanoma	0

Example 8: Generation of SAD-specific Immunoreagents

A SAD-specific antisera was raised in rabbits against a KLH-conjugated synthetic peptide derived from the C-terminal region of SAD (amino acids 478 to 488 of SEQ ID NO:35) with a C to S substitution at position 486 essentially as described in Gentry and Lawton, Virology 152:421, 1984.

Example 9: Recombinant Expression of SAD10 Construction Of Vectors

Expression constructs were generated by PCR-based mutagenesis in which a BamHI site was introduced upstream of the initiating Met giving a 5' untranslated sequence of 5'-GGATCCCCGGACC-3' (SEQ ID NO:51). An N-terminal hexahistidine tagged construct was also created by PCR with the coding sequence for MRGSHHHHHH (SEQ ID NO:52) (ATGAGAGGATCGCATCACCATCACCATCAC, SEQ ID NO: 53) followed by the second nucleotide of the SAD coding sequence (a glutamate). Proteins tagged with this sequence can be recognized by the RGS•His Antibody (QIAGEN Inc.) and affinity purified with Ni-NTA resin (QIAGEN Inc.). These constructs were cloned into the 5'-BamHI and 3'-EcoRI sites of pBluescriptSK+ (Stratagene U.S.A.) and the 5'-BamHI and 3'-XhoI sites of the mammalian expression pcDNA3 (Invitrogen) for transient expression analysis.

The SpeI-XhoI full length SAD constructs were also cloned from pBluescriptSK+ (Stratagene U.S.A.) into the yeast expression vector pRSP (Superti-Furga et al., EMBO J. 12, 2625-2634). This vector contains a thiamine-repressible promoter in a shuttle vector for inducible expression in Schizosaccharomyces pombe. This system has been useful in studies of SRC family members for testing negative regulation by CSK, screening for additional regulators, and purifying recombinant

protein (Superti-Furga et al., EMBO J. 12, 2625-2634; Superti-Furga et al., Nature Biotech. 14, 600-605).

Transient Expression of SAD in Mammalian Cells

5 The pcDNA3 expression plasmids (5 µg DNA/60 mm plate) containing the unmodified and hexahistidine-tagged SAD genes were introduced into 293 cells with lipofectamine (Gibco BRL). After 48 hours, the cells were harvested in 0.25 mL RIPA (20 mM Tris-Cl pH 7.5, 150 mM NaCl, 1% Triton X-100, 1% deoxycholate,
10 0.1% SDS, 1mM DTT, 1 mM sodium vanadate, 1 mM phenylmethylsulfonyl fluoride, 2 µg/mL aprotinin, 1 µg/mL leupeptin, and 25 µg/mL trypsin inhibitor). Sample aliquots were resolved by SDS polyacrylamide gel electrophoresis (PAGE) on 10% acrylamide gels and electrophoretically transferred to
15 nitrocellulose. Non-specific binding was blocked by preincubating blots in Blotto (Tris buffered saline containing 5% w/v non-fat dried milk and 0.1% v/v Tween-20), and recombinant protein was detected using affinity-purified SAD-specific polyclonal antibody and peroxidase-linked secondary antibody
20 with the ECL kit (Amersham Life Science). Hexahistidine tagged protein was also detected using RGS•His Antibody (QIAGEN Inc.). Phosphotyrosine-containing proteins were detected by Western blotting with monoclonal antibody 4G10 (Upstate Biotechnology) with 3% BSA as the blocking agent.

25 Affinity purified anti-peptide antibody raised against the C-terminus of SAD (see Example 8) recognized a specific ~55 kDa protein in transfected 293 cells with greater than 90% of the expressed protein being RIPA-insoluble. This molecular weight is consistent with the molecular weight predicted based on SAD's
30 primary amino acid sequence (54,510 kD). SAD-transfected cells contain a prominent approximately 55 kDa tyrosine phosphorylated protein that is absent in vector controls. The

phosphorylated protein is most likely SAD itself because the band is clearly detected in IP-Westerns using anti-SAD crosslinked to protein A beads and 4G10 as the blotting antibody although anti-SAD only inefficiently immunoprecipitates.

Expression of Recombinant SAD in Schizosaccharomyces Pombe

S. pombe was used to express recombinant SAD as an approach to studying its function and regulation since this expression system has proven useful for studying Src family members (Superti-Furga et al., EMBO J. 12, 2625-2634; Superti-Furga et al., Nature Biotech. 14, 600-605). S. pombe strain SP200 (h-s leu1.32 ura4 ade210) was grown as described and transformations with pRSP expression plasmids were done by the lithium acetate method (Moreno et al., 1991; Superti-Furga et al., EMBO J. 12, 2625-2634). Cells were grown in the presence of 1 uM thiamine to repress expression from the nmt1 promoter or in the absence of thiamine to induce expression.

Under derepressing conditions, SAD-expressing strains show no growth defect compared to vector controls in either liquid culture or solid media. This result contrasts with the toxicity caused by expression of several other tyrosine kinases including Src and Frk. SAD protein can be detected in these strains as a weak band on Western blots using the polyclonal antibody against the C-terminus. On anti-phosphotyrosine Western blots, SAD itself is the only detectable phosphotyrosine-containing protein, however in the presence of pervanadate, cellular proteins are also phosphorylated. This observation contrasts with the results seen for Src and MKK3 which phosphorylate many yeast proteins even in the absence of phosphatase inhibitors. These findings suggest that SAD exhibits relatively limited substrate specificity and

autophosphorylates itself. These results are consistent with the transient expression experiments in 293 cells.

Example 10: Assay for SAD Kinase Activity

5 The example below describes an in vitro assay for SAD kinase activity. The assay is useful for the identification of modulators of SAD activity.

Materials And Methods

10 S. pombe expressing hexahistidine-tagged SAD were harvested by centrifugation and lysed by the glass bead method (Superti-Furga et al., EMBO J. 12, 2625-2634) in NP-40 lysis buffer (50 mM Tris-Cl pH 7.5, 150 mM NaCl, 1% NP-40, 5 mM 2-mercaptoethanol, 1 mM sodium vanadate, 1 mM
15 phenylmethylsulfonyl fluoride, 2 µg/mL aprotinin, 1 µg/mL leupeptin, and 25 µg/mL trypsin inhibitor). Immunoprecipitations were done by mixing yeast extract (100 µg total protein in 100 µL NP-40 lysis buffer) with 0.6 µg the RGS•His Antibody (QIAGEN Inc.) and 10 µL Protein A/G agarose (Upstate
20 Biotechnology) for 3 hrs at 4 °C. IP complexes were washed four times in 1 mL lysis buffer and once in 1 mL kinase buffer (20 mM Na-HEPES pH 7.5, 10 mM MnCl₂, 2 mM 2-mercaptoethanol, and 10 µM sodium vanadate). Kinase assays were for 10 min at 30 °C in
25 40 µl kinase buffer containing 15 µM ATP, 0.5 uCi γ-³²P-ATP, and either 3 µg denatured enolase or 10 µg poly-Glu-Tyr (4:1) as the substrate. Extracts were assayed using 2-10 µg total protein per reaction and IP complexes were assayed using 5 µl Protein A/G beads per assay. Reactions were terminated by the addition of SDS sample buffer and the samples were resolved on
30 an 10% SDS polyacrylamide gel and visualized by autoradiography.

Results

SAD was able to phosphorylate both denatured enolase and poly-Glu-Tyr in vitro. Phosphorylation of both substrates was detected in crude yeast lysates expressing SAD but not in lysates from vector control strains. In addition, anti-His IP complexes from SAD-expressing strains but not control strains phosphorylated both denatured enolase and poly-Glu-Tyr.

10 Example 11: Isolation Of cDNA Clones Encoding PTP05 and PTP10

The example below describes the isolation and identification of new PTP sequences from primary murine fat and rat basal forebrain and the subsequent cloning of a full-length PTP05 sequence. Also described are probes useful for the detection of PTP05 and/or PTP10 in cells or tissues.

Materials and Methods:

Total RNAs were isolated using the Guanidine Salts/Phenol extraction protocol of Chomczynski and Sacchi (P. Chomczynski and N. Sacchi, Anal. Biochem. 162, 156 (1987) from ob/ob mouse fat and, separately, rat basal forebrain. These RNAs were used to generate single-stranded cDNA using the Superscript Preamplification System (GIBCO BRL, Gaithersburg, MD.; Gerard, et al, FOCUS 11:66, 1989) under conditions recommended by the manufacturer. A typical reaction used 10 μ g total RNA with 1.5 μ g oligo(dT)₁₂₋₁₈ in a reaction volume of 60 μ L. The product was treated with RNaseH and diluted to 100 μ L with H₂O. For subsequent PCR amplification, 1-4 μ L of this sscDNA was used in each reaction.

Degenerate oligonucleotides were synthesized on an Applied Biosystems 394 DNA synthesizer using established

100

phosphoramidite chemistry, precipitated with ethanol and used unpurified for PCR. The sequence of the degenerate oligonucleotide primers follows:

PTPDFW = 5'-GAYTTYTGGVRNATGRTNTGGGA- (sense) (SEQ ID NO: 17) and

PTPHCSA = 5'-CGGCCSAYNCCNGCNSWRCARTG -3' (antisense) (SEQ ID NO: 18).

These primers were derived from the peptide sequences DFWMXW(E/D) (SEQ ID NO: 19) (sense strand from PTP catalytic domain) and HCXAGXG (SEQ ID NO: 20) (antisense strand from PTP catalytic domain), respectively. The standard UIPAC designations for degenerate residue designations are: N = A, C, G, or T; R = A or G; Y = C or T; V = A, C or G; W = C or T; S = C or G; M = A or C; and H = A, C or T.

PCR reactions were performed using degenerate primers applied to the single-stranded cDNA listed above. The primers were added at a final concentration of 5 μ M each to a mixture containing 10 mM Tris·HCl (pH8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each deoxynucleoside triphosphate, 0.001% gelatin, 1.5 U AmpliTaq DNA Polymerase (Perkin-Elmer/Cetus), and 1-4 μ L cDNA. Following 3 min denaturation at 95 °C, the cycling conditions were 94 °C for 30 sec, 50 °C for 1 min, and 72 °C for 1 min 45 sec for 35 cycles. PCR fragments migrating between 350-400 bp were isolated from 2% agarose gels using the GeneClean Kit (Biol01), and T-A cloned into the pCRII vector (Invitrogen Corp. U.S.A.) according to the manufacturer's protocol.

Colonies were selected for mini-plasmid DNA-preparations using Qiagen columns and the plasmid DNA was sequenced using cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction products were run on an ABI Prism 377 DNA Sequencer, and analyzed using the BLAST alignment algorithm (Altschul, S.F. et

al., J. Mol. Biol. 215:403-10). Several copies of a clone encoding a novel PTP (R90-2-22), designated SuPTP05, was isolated from murine adipose tissue. A related clone, PTP10, was isolated from rat basal forebrain.

5 To obtain full-length cDNA encoding the novel phosphatase PTP05, RACE (rapid amplification of cDNA ends) was performed with sense or anti-sense oligonucleotides derived from the original PCR fragments. Marathon-Ready cDNA (Clontech, Palo Alto, CA) made from mouse testis was used in the RACE reactions
10 with the following primers:

RACE primers:

5'-CACCGTTCGAGTATTTTCAGATTGTGAAGAAGTCC-3' (6595) (SEQ ID NO:21),
5'-GGACTTCTTCACAATCTGAAATACTCGAACGGTG-3' (6596) (SEQ ID NO:22),
15 5'-CCGTTATGTGAGGAAGAGCCACATTACAGGACC-3' (6599) (SEQ ID NO:23),
5'-GGTCCTGTAATGTGGCTCTTCCTCACATAACGG-3' (6600) (SEQ ID NO:24),
AP-1, and AP-2 (Clontech).

RT-PCR primers for PTP05 sequencing:

20 5'-CACCGTTCGAGTATTTTCAGATTGTGAAGAAGTCC-3' (6595) (SEQ ID NO:21),
5'-GGTCCTGTAATGTGGCTCTTCCTCACATAACGG-3' (6600) (SEQ ID NO:24).

Isolated cDNA fragments encoding SuPTP05 were confirmed by DNA sequencing and subsequently used as probes for the screening of a murine testis cDNA library.

25 Two murine testis cDNA libraries (12apII, Stratagene, La Jolla, CA and lgt10, Clontech), were screened to isolate full-length transcripts encoding PTP05. The 5' or 3'-RACE fragments were ³²P-labeled by random priming and used as hybridization probes at 2x10⁶ cpm/mL following standard techniques for library
30 screening. Pre-hybridization (3 hrs) and hybridization (overnight) were conducted at 42 °C in 5X SSC, 5 X Denhart's solution, 2.5% dextran sulfate, 50 mM Na₂PO₄/NaHPO₄ [pH 7.0], 50% formamide with 100 mg/mL denatured salmon sperm DNA.

Stringent washes were performed at 65 °C in 0.1X SSC and 0.1% SDS. Several overlapping clones were isolated and found to span the collective sequences of the PCR fragment (R90-2-22) and the RACE products. The final sequence was verified by sequencing of both strains using a cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction products were run on an ABI Prism 377 DNA Sequencer. A full-length PTP10 clone can be obtained using the same techniques.

10 Results:

The primary murine PTP05 transcript is 1785 nucleotides and encodes a predicted polypeptide of 426 amino acids with a predicted molecular weight of 49122 daltons (SEQ ID NO:3 and SEQ ID NO:11). The PTP05 coding sequence is flanked by a 198 nucleotide 5'-untranslated region and a 279 nucleotide 3'-untranslated region ending with a poly(A) tail. There are inframe stop codons in all three frames upstream of the primary open reading frame. The ATG beginning at nucleotide position 199 conforms to the Kozak consensus for an initiating methionine. One clone (#6.1) contains an insertion of 111 bp at nucleotide 328 resulting in an addition 37 amino acids added inframe to the coding sequence. A second clone (#10.1) has a deletion of 93 bp beginning at nucleotide 1415, resulting in a frame-shift and premature termination. Upstream of the 198bp 5'UTR, the numerous clones diverge into 2 groups, extending the 5'UTR an additional 98-153 bp. Furthermore, one clone (#15.3) lacks the polyA tail at nucleotide 1758 extends the 3' UTR by another 300 nucleotides.

The amino acid sequence shows no signal sequence or a transmembrane domain, and PTP05 is therefore predicted to be an intracellular protein. The N-terminal domain of murine PTP05 extends from amino acid 1 to 187 and is unique, i.e. contains no significant homology to any protein in the non-redundant

protein database. The non-redundant protein database consists of peptide sequences from GenBank Genpept, PIR, and SwissProt. There is a single protein tyrosine phosphatase catalytic domain extending from amino acids 188-420. The catalytic domain
5 shares a relatively low level of identity at the amino acid level (40-47%) to PTPs from 5 distinct families: ZPEP (mouse) (46.7%), PTP-BAS (human) (45.6%), DEP (human) (40.5%), PTP-g (human) (40.6%), suggesting that it represents a new family of PTPs. The C-terminal tail of PTP05 extends beyond the cata-
10 lytic domain from amino acids 421-426 and is not homologous to other protein tyrosine phosphatases. Motifs found in the cytoplasmic domain of other mammalian PTPs that are absent from PTP05 include: SH2, Talin/Ezrin-like, PEST, GLGF, and Retinaldehyde-binding protein domains. Owing to its divergent
15 catalytic domain and absence of well-known non-catalytic motifs, we have designated PTP05 as a new and distinct family of protein tyrosine phosphatases.

An alternative form of murine PTP05 contains an insertion of 111-bp in the N-terminal coding region, extending the
20 sequence by 37 aa (SEQ ID NO:4 and SEQ ID NO:12). This 1,896 bp "long" form of murine PTP05 encodes a polypeptide of 463 amino acids with a predicted molecular weight of 53716 daltons. The insertion is located at amino acid positions 44-80 and is not significantly homologous to other proteins in the non-
25 redundant protein database.

A third form of PTP05 has a deletion of nucleotides 1415-1507 resulting in a frame shift and C-terminal truncation leading to an alternate sequence from amino acids 406-412 (SEQ ID NO:5 and SEQ ID NO:13). The 1,692 bp "C-trunc" murine PTP05
30 encodes a polypeptide of 412 amino acids with a predicted molecular weight of 47233 daltons.

The rat PTP10 clone shares 92% identity at the DNA level (320 nucleotides) and 85% amino acid identity at the protein

level (107 amino acids) with murine PTP05 (See Figure 1). The level of homology of the two catalytic domains suggests that PTP05 and PTP10 are distinct but related genes, and thus PTP10 is considered to be a second member of this new PTP family.

5 Partial sequences of rat PTP10 are shown in SEQ ID NO:6 (nucleic acid) and SEQ ID NO:14 (amino acid).

Example 12: Expression of PTP05

The example below shows the evaluation of PTP05 and PTP10 expression in normal murine tissues. A similar analysis can be done in human tissues using a human PTP05 or PTP10.

10

Materials and Methods:

A mouse normal tissue Northern blot containing 2 μ g polyA+ mRNA per lane from 8 different mouse adult tissues (lung, testis, brain, heart, liver, kidney, spleen, skeletal muscle) on a charge-modified nylon membrane was obtained from Clontech (#7762-1, Palo Alto, CA).

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The membrane was hybridized with randomly primed [a³²P]dCTP-labeled probe synthesized from a 241 bp EcoRI fragment of R90-2-22 (see above). Hybridization was performed at 42 °C overnight in 5X SSC, 2% SDS, 10X Denhardt's solution, 50% formamide, 100 μ g/mL denatured salmon sperm DNA with 1-2 x 10⁶ cpm/mL of ³²P-labeled DNA probe. The membrane was washed at room temperature in 2X SSC/0.05% SDS for 30 min and followed by 50 °C in 0.2X SSC/0.1% SDS for 30 min, and exposed overnight on Kodak XAR-2 film.

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A similar analysis was performed using the 320 bp rat PTP10 fragment as a probe of a rat normal tissue Northern blot.

RT-PCR Detection of Novel PTPs

Total RNA was isolated from fresh frozen mouse or rat (separately) tissues by centrifugation through a cesium chloride cushion. Twenty µg of total RNA was reverse transcribed with random hexamers and Moloney murine leukemia virus reverse transcriptase (Super-ScriptII, GIBCO BRL, Gaithersburg, MD). PCR was then used to amplify cDNA encoding SuPTP05. RT-PCR reactions lacking only the reverse transcriptase were performed as controls. PCR products were electrophoresed on 3% agarose gels, visualized by ethidium bromide staining and photographed on a UV light box. The intensity for a 161-bp fragment specific to murine PTP05 were compared among different RNA samples. A rating of 3 represents large quantities of PTP05 transcript identified by Northern blot analysis while a rating of 0 represents little or none of the transcript was detected.

Results:

By Northern analysis, a single murine PTP05 mRNA transcript of approximately 3.4 kb was identified, and found to be exclusively expressed in the testis. The lung, brain, heart, liver, kidney, spleen, skeletal muscle samples were negative. PTP10 hybridized to a slightly smaller band and was also found only in the testis in this analysis. Northern analysis identified two rat PTP10 mRNA transcripts of approximately 3.3 kb and 1.8 kb, exclusively expressed in the testis. The rat heart, brain, spleen, lung, liver, skeletal muscle, and kidney samples were negative.

RT-PCR with gene specific primer-pairs showed that expression of the transcripts encoding PTP05 confirmed the results from Northern analysis and also detected low levels in adipose, kidney, small intestine, and cells/tissues of hematopoietic or immune origin including spleen, thymus, lymph node, bone marrow, and peripheral blood lymphocytes). RT-PCR

with rat PTP10 gene specific primers confirmed the results from the Northern analysis, detecting a strong signal only in rat testis ssDNA and not in templates corresponding to rat skeletal muscle, heart, kidney, spleen, adrenal gland, lung, liver, intestine, uterus, spinal cord, brain, cortex and ovary.

The relatively selective expression of PTP05 in cells of hematopoietic or immune origin suggests a potential involvement in immune regulation including T and B cell survival, differentiation or co-stimulation, and/or inflammatory, immunosuppressive or autoimmune disorders. Additionally, expression in adipose tissue (also the source from which PTP05 was originally isolated) suggests a possible role in metabolic disorders such as diabetes.

Example 13: Recombinant Expression Of PTP05

The following example illustrates the construction of vectors for expression of recombinant PTP05 and the creation of recombinant cell lines expressing PTP05. Similar vectors and recombinant cell lines can be generated using PTP10 and the techniques described herein.

Construction of Expression Vectors

Expression constructs were generated by PCR-assisted mutagenesis in which the entire coding domain of PTP05 was tagged on its carboxy-terminal end with the hemophilus influenza hemagglutinin (HA) epitope YPYDVPDYAS (SEQ ID NO:55) (Pati, supra). This construct were introduced into two mammalian expression vectors: pLXSN (Miller, A.D. & Rosman, G.J., Biotechniques 7, 980-988, 1989) for the generation of virus producing lines; and pRK5 for transient expression in mammalian cells.

Dominant negative PTP05 constructs were also made in both pLXSN and pRK5 by mutation of the invariant Cys in the

conserved His-Cys-Ser-Ala-Gly motif (SEQ ID NO:56) to an Ala by PCR mutagenesis.

The entire PTP05 open reading frame excluding the initiating methionines was generated by PCR and ligated into pGEX vector for bacterial production of GST-fusion proteins for immunization of rabbits for antibody production. This vector contains the glutathione-S-transferase coding sequence followed by a polylinker for generating recombinant fusion proteins. The GST moiety comprises the N-terminal portion of the fusion protein.

Transient Expression in Mammalian Cells

The pRK5 expression plasmids (10 µg DNA/100 mm plate) containing the HA-tagged PTP05 gene can be introduced into COS and 293 cells with lipofectamine (Gibco BRL). After 72 hours, the cells were harvested in 0.5 mL solubilization buffer (20 mM HEPES pH 7.35, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 1 µg/mL aprotinin). Sample aliquots were resolved by SDS polyacrylamide gel electrophoresis (PAGE) on 15% acrylamide/0.5% bis-acrylamide gels and electrophoretically transferred to nitrocellulose. Non-specific binding was blocked by preincubating blots in Blotto (phosphate buffered saline containing 5% w/v non-fat dried milk and 0.2% v/v nonidet P-40 (Sigma)), and recombinant protein was detected using a murine Mab to the HA decapeptide tag. Alternatively, recombinant protein can be detected using various PTP05-specific antisera.

Generation of Virus Producing Cell Lines

pLXSN recombinant constructs containing the PTP05 gene were transfected into an amphotropic helper cell line PA317 using CaCl₂ mediated transfection. After selection on G418,

the cells were plated on normal media without G418 (500 µg/mL). Supernatants from resistant cells were used to infect the ecotropic helper cell line GP+E86, and cells again selected on G418. Resistant cells were again taken off G418, and the
5 supernatants harvested every 8-12 hours and pooled as virus stock. Redemann et al., 1992, Mol. Cell. Biol. 12: 491-498. Viral stock titers were typically $\sim 10^6$ /mL.

Stable Expression in Mammalian Cells

10 NIH-3T3, and BALB/3T3 cells were grown in 100 mm plates with DMEM (Gibco) containing 10% fetal calf serum (FCS). The cells were superinfected with the PTP05 retrovirus by adding approximately 3 mL viral supernatant to 15 mL culture media for approximately 24 hours. Cells expressing the retroviral
15 constructs were then selected by growth in DMEM/10% FCS supplemented with 500 µg/mL G418.

Example 14: Generation Of Anti-PTP05 Antibodies

PTP05-specific immunoreagents were raised in rabbits
20 against a pool of three KLH-conjugated synthetic peptides corresponding to unique sequences present in human PTP04. The peptides (see below) were conjugated at the C-terminal residue with KLH.

Peptides used for immunizing rabbits:

25 PTP05:

peptide 433A - MSSPRKVRGKTGRDNDEEEGNSGNLNLNRN (SEQ ID
NO:57)

peptide 431A - SPVLSGSSRLSKDTETSVSEKELTQLAQI (SEQ ID
NO:58) and

30 peptide 432A - WDVSDRSLRNRWNSMDSETAGPSKTVSPV (SEQ ID
NO:59).

Additional immunoreagents were generated by immunizing rabbits with a purified preparation of a GST-fusion protein containing the entire coding region of PTP05. The GST-fusion proteins were produced in DH5-alpha E. coli bacteria as described in Smith, et al Gene 67:31, 1988. Bacterial protein lysates were purified on glutathione-sepharose matrix as described in Smith, et al., supra.

Example 15: Assay for PTP05 Activity

Materials and Methods:

Recombinant wild-type and dominant negative (signaling incompetent) PTP05 (see Example 13, supra) were purified from bacteria as GST-fusion proteins. Lysates were bound to a glutathione-sepharose matrix and washed twice with 1X HNTG, followed by one wash with a buffer containing 100 mM 2-(N-morpholino)ethanesulfonic acid (MES), pH 6.8, 150 mM NaCl, and 1 mM EDTA.

The assay for phosphatase activity was essentially done as described by Pei et al. (1993) using p-nitrophenolphosphate (PNPP) as a generic PTP substrate. Briefly, after the last washing step, reactions were started by adding 50 μ L Assay Buffer (100 mM MES pH 6.8, 150 mM NaCl, 10 mM DTT, 2 mM EDTA, and 50 mM PNPP) to the matrix bound proteins. Samples were incubated for 20 min. at 23 °C. The reactions were terminated by mixing 40 μ L of each sample with 960 μ L 1 N NaOH, and the absorbance of p-nitrophenol was determined at 450 nm. To control for the presence of PTP05 in the precipitates, the precipitates were boiled in SDS sample buffer and analyzed by SDS-PAGE. The presence of PTP05 was then detected by immunoblot analysis with anti-PTP05 antibodies.

Example 16: Isolation Of cDNA Clones Encoding ALP

The example below describes the isolation and identification of a new PTP sequence from mouse tissues and the subsequent cloning of a full-length human ALP. Also described are probes useful for the detection of ALP in cells or tissues.

Materials and Methods:

Total RNAs were isolated using a commonly known guanidine salts/phenol extraction protocol from normal mouse fat and rat pituitary. Chomczynski & Sacchi, 1987, Anal. Biochem. 162: 156. These RNA extracts were used to generate single-stranded cDNA using the Superscript Pre-amplification System (GIBCO BRL, Gaithersburg, MD.; Gerard et al., 1989, FOCUS 11: 66) under conditions recommended by the manufacturer. a typical reaction used 10 µg total RNA with 1.5 µg oligo(dT)₁₂₋₁₈ in a reaction volume of 60 µL. The product was treated with RNaseH and diluted to 100 µL with H₂O. For subsequent PCR amplification, 1-4 µL of this ssDNA was used in each reaction.

Degenerate oligonucleotides were synthesized on an Applied Biosystems 394 DNA synthesizer using established phosphoramidite chemistry, precipitated with ethanol and used unpurified for PCR. The sequence of the degenerate oligonucleotide primers were as follows:

PTPDFW = 5'-GAYTTYTGGVRNATGRTNTGGGA-3' (SEQ ID NO:17)
PTPHCSA = 5'-CGGCCSAYNCCNGCNSWRCARTG-3' (SEQ ID NO:18)
PTPYINA = 5'-ATCCCCGGCTCTGAYTAYATHMAYGC-3' (SEQ ID NO:60)

These primers were derived from the peptide sequences DFWXMXW(E/D) (SEQ ID NO:19) (sense strand from PTP catalytic region) and HCXAGXG (SEQ ID NO:20) (antisense strand from PTP catalytic region), and IPGSDYI(N/H)A (SEQ ID NO:61) respectively. The standard UIPAC designations for degenerate residue

designations are: N = A, C, G, or T; R = A or G; Y = C or T; V = A, C or G; W = C or T; S = C or G; M = A or C; and H = A, C or T.

5 PCR reactions were performed using degenerate primers applied to the single-stranded cDNA listed above. The primers were added at a final concentration of 5 μ M each to a mixture containing 10 mM TrisHCl (pH8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each deoxynucleoside triphosphate, 0.001% gelatin, 1.5 U AmpliTaq DNA Polymerase (Perkin-Elmer/Cetus), and 1-4 μ L cDNA. 10 Following 3 min denaturation at 95°C, the cycling conditions were 94 °C for 30 s, 50 °C for 1 min, and 72°C for 1 min 45 s for 35 cycles. PCR fragments migrating between 350-400 bp were isolated from 2% agarose gels using the GeneClean Kit (Biol01), and T-A cloned into the pCRII vector (Invitrogen Corp. U.S.A.) 15 according to the manufacturer's protocol.

Colonies were selected for mini plasmid DNA-preparations using Qiagen columns and the plasmid DNA was sequenced using cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction 20 products were run on an ABI Prism 377 DNA Sequencer, and analyzed using the BLAST alignment algorithm. Altschul et al., J. Mol. Biol. 215: 403-410. A single clone encoding a novel PTP (S50-151), designated murine ALP, was isolated from murine adipose tissue using degenerate oligonucleotides PTPDFW (SEQ ID NO: 17) and PTPHCSA (SEQ ID NO:18), and a related rat ALP clone 25 was isolated from rat pituitary using degenerate oligonucleotides PTPYINA (SEQ ID NO:60) and PTPHCSA (SEQ ID NO:18).

To isolate a full-length human ALP a human cDNA library 30 was constructed in lambda ZapII (Stratagene, La Jolla, CA) from polyA+ RNA isolated from the human neuroblastoma cell line IMR32. The library was screened to isolate full-length

transcripts encoding ALP. The murine ALP fragment was ³²P-labeled by random priming and used as a hybridization probe at 2x10⁶ cpm/mL following standard techniques for library screening. Pre-hybridization (3 h) and hybridization (overnight) were conducted at 42 °C in 5X SSC, 5 X Denhart's solution, 2.5% dextran sulfate, 50 mM Na₂PO₄/NaHPO₄ [pH 7.0], 50% formamide with 100 mg/mL denatured salmon sperm DNA. Stringent washes were performed at 65 °C in 0.1X SSC with 0.1% SDS. Multiple clones were isolated and one 4.5 kb clone spanned the entire coding region of ALP. The final sequence was verified by sequencing of both strands using a cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction products were run on an ABI Prism 377 DNA Sequencer.

Results:

The 4,456 bp human ALP nucleotide sequence encodes a polypeptide of 1,274 amino acids. The amino acid sequence shows no signal sequence or a transmembrane domain and is therefore an intracellular protein. The N-terminal end extends from amino acids 1-857 and contains several putative tyrosine phosphorylation sites and a proline-rich region (30.6% prolines) from amino acids 353-777. This proline-rich region is distantly related to plant extensin proteins (30.2% amino acid identity with Zea mays extensin-like protein GB:Z34465 using Smith-Waterman alignment) and may represent a protein interaction domain as well as the site for interaction with proteins containing SH3 motifs. The C-terminal tail of ALP extends from amino acid 1097-1274 and contains a proline/serine rich region (45.6% serines plus prolines from amino acids 1101-1214) resembling a PEST motif. This region also could serve as a target for binding proteins via their SH3 motifs.

The catalytic domain extends from amino acids 858-1096 and shares 32-37% amino acid identity to PTPs from multiple subfamilies: TC-PTP (P17706: 37.1%) PTP-BAS (D21209: 32.9%), PTP α (M34668: 34.2%), PTP β (P23467: 34.2%), PTP σ (A49104: 33.2%), PTP1B (P20417: 34.9%), suggesting that it represents a new family of PTPs. While all other cytoplasmic PTPs have their catalytic domain at either the N- or C-terminal portion of the protein, ALP has a central catalytic domain flanked by large N- and C-terminal domains. Its catalytic domain conserves most of the invariant residues present in other PTPs, but does have several atypical amino acids. In ALP, the amino acid sequence HCSAG (SEQ ID NO:56), is changed to HCSSG (amino acid positions 1029-1033) (SEQ ID NO:75). This motif is in the catalytic site of the crystal structure of PTP1B and PTP α , and the Ala to Ser change may effect catalytic activity or specificity. ALP also has a change from WPD to WPE (amino acid positions 993 - 995) in its predicted surface loop of the catalytic domain. In PTP1B this Aspartate participates in a salt bridge and falls into the catalytic site on binding to a specific peptide substrate. This Asp to Glu alteration is also present in three other mammalian PTPs (PTPD1, PCP2, PTPS31).

Example 17: Expression Of ALP

The example below shows the evaluation of ALP expression in normal human tissues and in a wide variety of cancers.

Materials and Methods:

Northern blots were prepared by running 20 μ g total RNA per lane isolated from 60 different tumor cell lines (HOP-92, EKVX, NCI-H23, NCI-H226, NCI-H322M, NCI-H460, NCI-H522, A549, HOP-62, OVCAR-3, OVCAR-4, OVCAR-5, OVCAR-8, IGROV1, SK-OV-3, SNB-19, SNB-75, U251, SF-268, SF-295, SF-539, CCRF-CEM, K-562,

MOLT-4, HL-60, RPMI 8226, SR, DU-145, PC-3, HT-29, HCC-2998, HCT-116, SW620, Colo 205, HTC15, KM-12, UO-31, SN12C, A498, CaKi1, RXF-393, ACHN, 786-0, TK-10, LOX IMVI, Malme-3M, SK-MEL-2, SK-MEL-5, SK-MEL-28, UACC-62, UACC-257, M14, MCF-7, MCF-7/ADR RES, Hs578T, MDA-MB-231, MDA-MB-435, MDA-N, BT-549, T47D). (obtained from Nick Scudero, National Cancer Institute, Developmental Therapeutics Program, Rockville, MD). The total RNA samples were run on a denaturing formaldehyde 1% agarose gel and transferred onto a nitrocellulose membrane (BioRad, CA). Additional human normal tissue Northern blots containing 2 μ g polyA+ mRNA per lane from 16 different human normal tissues (thymus, lung, colon, testis, brain, heart, liver, pancreas, kidney, spleen, uterus, prostate, skeletal muscle, PBLs, placenta, small intestine) on charge-modified nylon membranes (multiple tissue blots #7760-1 and #7766-1, Clontech, Palo Alto, CA) were also hybridized.

Nitrocellulose membranes for the total RNA samples were hybridized with randomly primed [γ - 32 P]dCTP-labeled probes synthesized from a 1 kb fragment of EcoRI-NotI of ALP. Hybridization was performed overnight at 42 °C in 4X SSPE, 2.5X Denhardt's solution, 50% formamide, 200 μ g/mL denatured salmon sperm DNA, 100 μ g/mL yeast tRNA (Boehringer Mannheim, IN), 0.2% SDS with 5×10^6 cpm/mL of [γ - 32 P]dCTP-labeled DNA probe on a Techne Hybridizer H-1. The blots were washed with 2X SSC, 0.1% SDS, at 65 °C for 20 min twice followed by 0.5 X SSC in 0.1% SDS at 65 °C for 20 min. The blots were exposed to a phospho-imaging screen for 24 hours and scanned on a Molecular Dynamics Phosphoimager SF.

For Clontech nylon-membrane blots, hybridization was performed at 42 °C overnight in 5X SSC, 2% SDS, 10X Denhardt's solution, 50% formamide, 100 μ g/mL denatured salmon sperm DNA with $1-2 \times 10^6$ cpm/mL of [γ - 32 P]dCTP-labeled DNA probe. The

blots were washed at room temperature in 2X SSC/0.05% SDS for 30 min and followed by at 50 °C in 0.2X SSC/0.1% SDS for 30 min, and exposed for 48 hours on Kodak XAR-2 film.

For analysis of expression using reverse-transcriptase-PCR detection, total RNA was isolated from various cell lines or fresh frozen tissues by centrifugation through a cesium chloride cushion. 20 µg of total RNA was reverse transcribed with random hexamers and Moloney human leukemia virus reverse transcriptase (Super-ScriptII, GIBCO BRL, Gaithersburg, MD). PCR was then used to amplify cDNA encoding ALP. Reverse transcriptase PCR (RT-PCR) reactions lacking only the reverse transcriptase were performed as controls. PCR products were electrophoresed on 3% agarose gels, visualized by ethidium bromide staining and photographed on a UV light box.

The intensity of the fragment specific to ALP were compared among different RNA samples. A rating of 4 represents large quantities of ALP transcript while a rating of 0 represents little or none of the transcript was detected. It should be noted that detection of proteins by RT-PCR indicates a relatively higher abundance than detection by Northern blot as the RT-PCR technique utilizes total RNA whereas Northern blot analysis is performed using an enriched RNA source (mRNA).

Results:

A single ALP mRNA transcript of approximately 5.0 kb was visualized by Northern analysis. This transcript was identified in most of the normal tissue samples tested. However, the Northern analysis results shown in the Table 1 illustrate that the relative abundance of ALP mRNA is quite divergent. In normal tissues, ALP was identified in highest quantities in pancreas, followed by heart, testis, and skeletal muscle. Lower levels of the ALP transcript were identified in placenta,

thymus, lung, brain, liver, spleen, uterus, prostate and small intestine. None of the ALP transcript was detected in colon, kidney and peripheral blood leucocytes (PBLs). ALP expression was also detected in normal human adipocytes by RT-PCR methods.

5 In Northern blots of total RNA from human tumor cell lines, the ALP RNA transcript was most abundant in NCI-H226 (lung tumor), SK-OV-3 (ovarian tumor), and RPMI 8226 (leukemia) cell lines. The transcript was identified at lower amounts in SNB-19 (CNS tumor), SF-268 (CNS tumor), SN12C (kidney tumor),
10 SK-MEL-2 (melanoma), UACC-62 (melanoma), and UACC-257 (melanoma) cell lines. The ALP transcript was not detected in the remaining of 44 human tumor cell lines. A summary of expression of ALP is shown in Table 1 below.

Table 1

	Cell type	Origin	ALP
	Thymus	Normal tissue	0.5*
	Lung	Normal tissue	0.5*
5	Colon	Normal tissue	0*
	Testis	Normal tissue	2*
	Brain	Normal tissue	0.5*
	Heart	Normal tissue	2*
	Liver	Normal tissue	0.5*
10	Pancreas	Normal tissue	3*
	Kidney	Normal tissue	0*
	Spleen	Normal tissue	0.5*
	Uterus	Normal tissue	0.5*
	Prostate	Normal tissue	0.5*
15	Skeletal muscle	Normal tissue	2*
	PBLs	Normal tissue	0*
	Placenta	Normal tissue	1*
20	Small intestine	Normal tissue	0.5*
	NCI-H226	Lung tumor	4
	SK-OV-3	Ovarian tumor	3
	SNB-19	CNS tumor	2
	U251	CNS tumor	1
25	SF-268	CNS tumor	2
	RPMI 8226	Leukemia	3

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Cell type	Origin	ALP
HTC15	Colon tumor	1
UO-31	Colon tumor	1
SN12C	Kidney tumor	2
SK-MEL-2	Melanoma	2
SK-MEL-28	Melanoma	1
UACC-62	Melanoma	2
UACC-257	Melanoma	2
T47D	Breast tumor	1

* mRNA Northern blot.

ALP exhibits increased expression in tumor cells compared to their normal tissue counterparts. This differential expression suggests a possible dysregulation or involvement of ALP in development or maintenance of the transformed phenotype.

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Example 18: Recombinant Expression of ALP

The following example illustrates the construction of vectors for expression of recombinant ALP and the creation of recombinant cell lines expressing ALP.

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Construction of Expression Vectors

Expression constructs were generated by PCR-assisted mutagenesis in which the entire coding regions of ALP was introduced into the mammalian expression vectors pcDNAIII (Invitrogen) for transient expression analysis. Additional ALP constructs were made by oligonucleotide based PCR mutagenesis to convert atypical residues in the PTP-related domain back to the amino acids more commonly present in other catalytically active PTPs. These changes include: His to Tyr at amino acid 861 (See SEQ. ID. NO.:2); Ala to Gly at amino acid 902; Phe to trp at amino acid 941; Glu to Asp at amino acid 995; and Ser to Ala at amino acid 1032. Additional constructs containing paired mutations as above were generated for amino acid positions 941/1032 and 902/1032. These constructs were ligated into the pcDNAIII mammalian expression vector behind the CMV promoter.

The entire ALP open reading frame excluding the initiating methionines was generated by PCR and ligated into pGEX vector (Pharmacia Biotech, Upsala, Sweden) for bacterial production of GST-fusion proteins for immunization of rabbits for antibody production. This vector contains the glutathione-S-transferase coding sequence followed by a polylinker for generating recombinant fusion proteins. The GST moiety comprises the N-

30

terminal portion of the fusion protein. The various ALP mutants were also inserted into the pGEX vector for production of recombinant protein reagents.

5 Transient Expression in Mammalian Cells

The pcDNAIII expression plasmids (10 μ g DNA/100 mm plate) containing the wild-type and mutant forms of the ALP gene were introduced into 293 cells with lipofectamine (Gibco BRL). After 72 hours, the cells were harvested in 0.5 mL solubilization buffer (20 mM HEPES pH7.35, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM $MgCl_2$, 1 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 1 μ g/mL aprotinin). Sample aliquots were resolved by SDS polyacrylamide gel electrophoresis (PAGE) on 15%acrylamide/0.5% bis-acrylamide gels and electrophoretically transferred to nitrocellulose. Non-specific binding was blocked by preincubating blots in Blotto (phosphate buffered saline containing 5% w/v non-fat dried milk and 0.2% v/v nonidet P-40 (Sigma)), and recombinant protein was detected using antisera specific to the amino-terminal 352 residues (see below). Recombinant ALP protein migrated approximately 180 kDa, consistent with the predicted molecular weight of the 1274 amino acid protein.

Endogenous ALP was detected as a 200 kD protein in Western blots of lysates from a variety of tumor cell lines including human glioblastomas (U87MG, ATCC HTB 14; U118MG, ATCC HTB 15; U138MG, ATCC HTB 16; A172, ATCC CRL 1620; Hs683, ATCC HTB 138), rodent gliomas (C6, ATCC 107), rodent pituitary tumors (ATT20, ATCC CCL 89; GH3, ATCC CCL 82.1), human neuroblastomas (SKNMC, ATCC HTB 10; IMR 32, ATCC CCL 127), and rodent adrenal pheochromocytomas (PC12, ATCC CRL 1721). ALP protein could not be immunoprecipitated from the non-transformed cell line NIH 3T3 (ATCC CRL 1658).

It is unclear why native ALP protein appears to be larger (200 kDa) than recombinant ALP detected in transfected 293 cells (180 kDa). The difference could be the result of alternative RNA splicing, or a post-translational modification in the cell lines where it is endogenously expressed. Preliminary experiments indicate that ALP is phosphorylated on serine and threonine residues in transfected 293 cells. In addition, several tyrosine-phosphorylated proteins are associated with ALP since they are detected in Western blots using an anti-phosphotyrosine antibody following immunoprecipitation of endogenous ALP from human tumor cell lines such as IMR32 after treatments with the phosphatase inhibitor pervanadate.

Generation Of Virus Producing Cell Lines

pLXSN recombinant constructs containing the ALP gene are transfected into an amphotropic helper cell line PA317 using CaCl_2 mediated transfection. After selection on G418, the cells are plated on normal media without G418 (500 $\mu\text{g/mL}$). Supernatants from resistant cells are used to infect the ecotropic helper cell line GP+E86, and cells again selected on G418. Resistant cells are again taken off G418, and the supernatants harvested every 8-12 hours and pooled as virus stock. Redemann et al., 1992, Mol. Cell. Biol. 12: 491-498. Viral stock titers are typically $\sim 10^6/\text{mL}$.

Stable Expression In Mammalian Cells

NIH-3T3, BALB/3T3 or other suitable cells are grown in 100 mm plates with DMEM (Gibco) containing 10% fetal calf serum (FCS). The cells are superinfected with the ALP retrovirus by adding approximately 3 mL viral supernatant to 15 mL culture media for approximately 24 hours. Cells expressing the

retroviral constructs are then selected by growth in DMEM/10% FCS supplemented with 500 µg/mL G418.

Example 19: Generation Of Anti-Alp Antibodies

5 ALP-specific immunoreagents were generated by immunizing rabbits with the bacterially expressed N-terminal 352 amino acid portion of ALP expressed as a GST-fusion protein. Fusion protein was affinity purified using glutathione-sepharose columns (Pharmacia). Polyclonal anti-serum against the N-termi-
10 nal portion of ALP was generated by repeatedly immunizing rabbits with the purified GST-fusions protein. Affinity-purified ALP antibody was obtained by binding serum IgG to ALP-GST-fusion protein immobilized on glutathione-sepharose and eluting with low pH and high salt.

15

Example 20: Assay For ALP Activity Assay For Modulators Of Catalytic Activity

Materials And Methods:

20 Recombinant wild-type and mutant ALP proteins are purified from bacteria as GST-fusion proteins. Lysates are bound to a glutathione-sepharose matrix and eluted with glutathione. The purified proteins are then washed with 2 x 1 mL HNTG, followed by one wash with 1 mL of a buffer containing 100 mM 2-(N-morpholino)ethansulfonic acid (MES), pH 6.8, 150 mM NaCl, and 1
25 mM EDTA. The assay for phosphatase activity is essentially done as described by Pei et al.(1993) using p-nitrophenolphosphate (PNPP) as a generic PTP substrate. Briefly, after the last washing step, reactions are started by
30 adding 50 µL Assay Buffer (100 mM MES pH 6.8, 150 mM NaCl, 10 mM DTT, 2 mM EDTA, and 50 mM p-nitrophenylphosphate) to the precipitates. Samples are incubated for 20 min. at 23 °C. The

reactions are terminated by mixing 40 μ L of each sample (without beads) with 960 μ L 1 N NaOH, and the absorbance of p-nitrophenol was determined at 450 nm. To control for the presence of ALP in the precipitates, the precipitates are
5 boiled in SDS sample buffer and analyzed by SDS-PAGE. The presence of ALP is then detected by immunoblot analysis with anti-ALP antibodies.

10 Example 21: A Consistent Method For Determination Of ZAP70 Kinase Activity.

The following protocol describes the reagents and procedures used to determine Zap70 protein kinase activities measuring phosphorylation of Band III-GST as readout. This
15 assay is used in search for inhibitors of Zap70.

Materials and Reagents

1. Baculovirus (PharMingen, CA) encoding for mutationally activated form of Zap70, in which a tyrosine residue
20 at position 492 is replaced with a phenylalanine residue (Y492F), containing a C-terminal HA tag and a N-terminal GST tag (GST-Zap70-HA) is used. The modified protein is termed GZH (i.e. Y492F GST-Zap70-HA = GZH).

2. Cell lysates: SF9 cells were infected with the GZH
25 virus at MOI of 10 for 96 hours. The cells were then washed once with PBS and lysed in lysis buffer. Insoluble material was removed by centrifugation (5 min. at 10 000 x g). Aliquots of lysates were frozen in dry ice/ethanol and stored at -80 °C until use.

30 3. Band III-GST: Band III-GST fusion protein (amino acid sequence: MEELQDYEDMMEEN (SEQ ID NO:62)) was expressed in XL1 Blue cells transformed with pGEX -2TK-Band III. Protein

expression was induced by addition of 0.5 mM IPTG while shaking the bacterial culture for 18 hours at 25 °C. Band III-GST by was purified by Glutathione affinity chromatography, Pharmacia, Alameda, CA

- 5 4. Biotinylated ITAM peptide 242 (ZETA-pY),
 Sequence: YQQGQNQLpYNELNLGRREEpYDVLDKRRGRD (SEQ ID NO:63)
 (Protein Chemistry Laboratory, SUGEN, INC., Redwood City, CA).
5. DMSO, Sigma, St. Louis, MO
6. 96 Well ELISA Plate: Corning 96 Well Easy Wash,
10 Modified Flat Bottom Plate. Catalog # 25805-96.
7. NUNC 96-well V-bottom polypropylene plates for
 dilution of compounds. Applied Scientific Catalog No.
 AS-72092
8. Streptavidin: Sigma S-8276
- 15 9. Purified Rabbit anti-GST antiserum. AMRAD catalog #
 9001605
10. Goat anti-Rabbit-IgG-HRP. Amersham Catalog No.
 V010301

20 Buffer solutions:

Lysis buffer:

10 mM Tris, pH 7.5
150 mM NaCl
1% NP40
25 1 mM PMSF
0.4 mM Na₃VO₄
2 mg/ml Leupeptin
2 mg/ml Aprotinin

Kinase buffer:

10 mM MgCl₂
10 mM MnCl₂
10 mM DTT
20 mM HEPES/Cl, pH 7.5
20 mM β-glycerophosphate
100 mM Na₃VO₄

30 Blocking buffer:

10 mM Tris, pH 7.5
100 mM NaCl
0.1% Tween 20

Wash buffer (TBST):

50 mM Tris, pH 7.5
150 mM NaCl
0.1% Tween 20

1% BSA

Procedure:

Preparation of Streptavidin Coated ELISA Plates:

- 5 Prepare borate buffer by titrating 0.1 M boric acid with 0.1 M sodium borate to pH 8.7. Add sodium azide to a final concentration of 0.05% and store at 4 °C. Prepare 1 mg/ml Streptavidin in borate buffer and store at 100 µL aliquots at -80 °C. Coat 0.1 µg/well Streptavidin in
- 10 100 µL of borate buffer at room temperature for 18 hours. Wash wells with 200 µL cold TBST twice. Invert the plate and blot the plate dry, cover with parafilm, and store at 4 °C for no more than one week. For longer storage, plates should be stored at -80 °C.

15

Preparation of phosphotyrosine antibody-coated ELISA plates:

Coat 1 µg/well 4G10 (Upstate Biotechnology, NY) in 100 µL of PBS overnight at 4 °C and block with 200 µL of blocking buffer for at least hour.

20

Kinase Assay Procedure

- Biotinated peptide 242 was bound to the ELISA Plate by incubating 1 µg/well in 100 µL PBS overnight at 4 °C with streptavidin coated ELISA Plate (see above). The wells were
- 25 blocked with 200 µL blocking buffer for 30 minutes at room temperature, after which the blocking buffer was removed by aspiration. Insect cell lysate containing the Zap70 fusion protein (GZH) was added (30 µg/well, volume adjusted to 100 µL/well with lysis buffer) and left to incubate at 4 °C for 2
- 30 hours. The lysate was removed by aspiration and the wells washed with TBST. Substrate and test compound (if any) were

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added and allowed to stand for 15 minutes (GST-Band III, 5 $\mu\text{g}/\text{well}$ in 90 μL final volume). The kinase reaction was started by the addition of 10 μL of 0.1 mM ATP per well for a final concentration of 10 μM . The 96 well plate was left for 30 minutes at room temperature (shaking) after which 90 μL of the reaction liquid was transferred to wells in a 96 well plate previously coated with an anti-phosphotyrosine antibody (UB40, Upstate Biotechnology, NY). This plate was allowed to stand for 30 minutes at room temperature, after which the liquid was removed and the wells washed with TBST. Rabbit anti-GST antibody was added (0.1 $\mu\text{g}/\text{well}$ in 100 μL blocking buffer) and incubated for 30 minutes at room temperature. The liquid was again removed and the wells washed with TBST. Goat anti-Rabbit-IgG-HRP was added at 1:40,000 dilution in 100 μL of blocking buffer for 30 minutes at room temperature, after which it was removed and the wells washed with TBST and developed with ABTS. The plate is then read in an ELISA plate reader at 410 nm. If the protein being tested is a captured protein, the reading from the ELISA plate reader can be related to the modulating activity of the test compound when it is compared with the activity of a control protein.

Example 22: Isolation And Characterization Of ALK-7

In order to isolate ALK-7, we designed degenerate oligonucleotides encoding amino acid motifs within kinase subdomains II and VI common to all known mammalian STK receptors. (Hanks and Hunter, FASEB J. 9:576-595, 1995) Subdomain II is at the N-terminus of the kinase domain and contains the invariant lysine residue that is essential for enzyme activity and is involved in ATP binding by interacting with the α - and β -phosphates of all kinases whose structure has been elucidated. Subdomain VI

is referred to as the catalytic loop and contains the consensus motif HRDLKXXN (SEQ ID NO:64). The Asp residue is involved in accepting the proton from the hydroxyl group during the phosphotransfer process key to all protein kinases. Based on comparison of all STK receptors, we designed degenerate oligonucleotide primers to these subdomains that would recognize both type I and type II STK receptors.

When this PCR strategy was applied to a human neuroblastoma cell line (SY5Y) ssDNA as a template, multiple copies of a novel DNA fragment (ALK-7) were isolated that exhibited significant homology to other STK receptors. The novel sequence was most similar to ALK-4 (Franzen, et al., Cell 75(4):681, 1993) and ALK-5 (ten Dijke, et al., Oncogene 8(10):2879, 1993) and was referred to as ALK-7.

Materials And Methods

Total RNAs were isolated using the Guanidine Salts/Phenol extraction protocol of Chomczynski and Sacchi (P. Chomczynski and N. Sacchi, Anal. Biochem. 162, 156 (1987) from normal human tissues, from regional sections of human brain, from cultured human tumor cell lines, and from primary neonatal rat sympathetic, motor, and sensory neuronal cells, as well as mesothalamic dopaminergic neurons.

These RNAs were used as templates to generate single-stranded cDNAs using the Superscript Preamplification System for First Strand Synthesis kit purchased from GibcoBRL (Life Technologies, U.S.A.; Gerard, G.F. et al. (1989), FOCUS 11, 66) under conditions recommended by manufacturer. A typical reaction used 10 µg total RNA or 2 µg poly(A)⁺ RNA with 1.5 µg oligo(dT)₁₂₋₁₈ in a reaction volume of 60 µL. The product was treated with RNaseH and diluted to 100 µL with H₂O. For

subsequent PCR amplification, 1-4 μ L of these ssCDNAs were used in each reaction.

Oligonucleotides were synthesized on an Applied Biosystems 394 DNA synthesizer using established phosphoramidite chemistry and were used unpurified after precipitation with ethanol. The degenerate oligonucleotide primers are:

STK1 = 5'-GARRARGT6GC6GT6AARRT6TT-3' (SEQ ID NO:65) (sense)

STK3- =

5'-TTRATRTC6CKRTG6GM6AT6GM6GGYTT-3' (SEQ ID NO:66) (antisense).

These primers were derived from the peptide sequences **E(K/E)VAVK(V/I)F** (SEQ ID NO:67) (sense strand from kinase subdomain II) and

KP(A/S)I(A/S)HRDIK (SEQ ID NO:68) (antisense strand from kinase subdomain VI), respectively. Degenerate nucleotide residue designations are: N = A, C, G, or T; R = A or G; Y = C or T; M = A or C; K = G or T; and 6 = Inosine. Using ALK1 as a template, these primers produce a product of 321 bp.

A PCR reaction was performed using primers STK1 and STK3- applied to the single-stranded sources listed above. The primers were added at a final concentration of 5 μ M each to a mixture containing 10 mM Tris HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 200 μ M each deoxynucleoside triphosphate, 0.001% gelatin, and 1.5 U AmpliTaq DNA Polymerase (Perkin-Elmer/Cetus), and 1-4 μ L cDNA. Following 3 min denaturation at 95°C, the cycling conditions were 94 °C for 30 s, 37 °C for 1 min, a 2 min ramp to 72 °C, and 72 °C for 1 min for the first 3 cycles, followed by 94 °C for 30 s, 50 °C for 1 min, and °C for 1 min 45 s for 35 cycles. PCR fragments migrating at ~320 bp were isolated from 2% agarose gels using GeneClean (Bio101), and T-A cloned into the pCRII vector (Invitrogen Corp. U.S.A.) according to the manufacturer's protocol.

Colonies were selected from mini plasmid DNA-preparations using Qiagen columns and the plasmid DNAs were sequenced using cycle sequencing dye-terminator kit with AmpliTaq DNA Polymerase, FS (ABI, Foster City, CA). Sequencing reaction products were run on an ABI Prism 377 DNA Sequencer, and analyzed using the BLAST alignment algorithm (Altschul, S.F. et al., J. Mol. Biol. 215:403-10). A novel clone (STKR6.22) was isolated by PCR with primers STK1 and STK3- on single-stranded cDNA from human SY5Y cells as a template. This clone was subsequently designated as a fragment of human ALK-7.

A lambda gt11 (Clontech, Palo Alto, CA) cDNA library was constructed using mRNA from a pool of nine whole human pituitary glands. Phage were screened on nitrocellulose filters with the random primed ³²P-labeled insert from STKR6.22 encoding human ALK-7 at 2x10⁶ cpm/mL in hybridization buffer containing 6xSSC, 1x Denhardt's reagent, 0.1% SDS, with 0.1 mg/mL denatured, fragmented salmon sperm DNA. After overnight hybridization at 65 °C, filters were washed in 0.1xSSC, 0.1% SDS at 65 °C. Full length cDNA clones were sequenced on both strands using manual sequencing with T7 polymerase and oligonucleotide primers (Tabor and Richardson, 1987, Proc. Natl. Acad. Sci., U.S.A. 84:4767-71).

Results

Two overlapping cDNA clones (P6 and P7), spanning 1794 nucleotides were isolated from a human pituitary library. This sequence contains an ATG at position 156 that conforms to the Kozak consensus for translational initiation and is followed by a 1,482 nucleotide open reading frame with the capacity to encode a polypeptide of 493 amino acids. There are no other initiation codons 5' to the ATG located at position 156. The coding region for human ALK-7 is flanked by 5' and 3' untranslated regions of 155 and 157, respectively. There is no

polyadenylated region although the 3' end of the sequence shown in SEQ ID NO:8 is noticeably AT-rich, a feature characteristic of sequences from 3'-untranslated regions. An additional cDNA clone (P4) extended an additional 1 kb 3' of this sequence.

5 DNA sequence determination was performed with dideoxy terminators using Sequenase 2.0. A primer walking strategy on both strands was used to confirm the complete nucleotide sequence. Oligonucleotide primers were made with an ABI 348 DNA synthesizer.

10 A Smith-Waterman search with the human ALK-7 gene sequence of the public nonredundant nucleic acid and EST databases revealed no identical matching sequences confirming that this is a novel human gene. The closest match to the human ALK-7 sequence (85% nucleic acid identity) is a recent entry (GenBank
15 ACC:U69702) which appears to be the rat orthologue of human ALK-7.

The 493 amino acid human ALK-7 sequence contains two hydrophobic regions from 1-25 and 114-138. (See SEQ ID NO:16) The first hydrophobic region meets the criteria of a signal
20 peptide domain, with a discriminant score of 5.76 using the method of McGeoch (D. J. McGeoch, Virus Research, 3, 271, 1985), and with a weight matrix score of +6.75 (threshold = 3.5) using the von Heijne algorithm (G. von Heijne, Nucl. Acids Res., 14, 4683, 1986). The second hydrophobic region generates
25 a likelihood score of -9.34, using the ALOM method of Klein et al. (P. Klein, M. Kanehisa, and C. DeLisi, Biochim. Biophys. Acta, 815, 468, 1985) to predict transmembrane domains. This algorithm predicts a maximal range of the transmembrane domain to be from aa 108-138.

30 Based on this analysis, ALK-7 is predicted to be a type Ia integral membrane protein with a molecular weight of 52.35 kD after cleavage of the N-terminal signal peptide.

Example 23: Expression Of ALK-7

Using both Northern blots and PCR analysis with the novel fragment originally cloned from SY5Y cells as described above as a probe, we screened RNAs using from a large number of tumor cell lines and multiple human tissues, demonstrating an apparent selectivity in expression of ALK-7 in neuronal cells from the pituitary and substantiate nigra.

Materials And Methods10 Northern Blot Analysis

Northern blots were obtained from Clontech (Palo Alto, CA) containing 2 µg polyA+ RNA from 16 different adult human tissues (spleen, thymus, prostate, testis, ovary, small intestine, colonic mucosa, heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, and peripheral blood leukocytes), and four different human fetal tissues (brain, lung, liver, and kidney), on a charge-modified nylon membrane. Additional Northern blots were prepared by running 20 µg total RNA on formaldehyde 1.2% agarose gel and transferring to nylon membranes.

Filters were hybridized with random prime [³²P]dCTP-labeled probes synthesized from the 320 bp insert from human ALK-7 clone STKR6.22. Hybridization was performed at 60 °C overnight in 6XSSC, 0.1% SDS, 1X Denhardt's solution, 100 mg/mL denatured herring sperm DNA with 1-2 x 10⁶ cpm/mL of ³²P-labeled DNA probes. The filters were washed in 0.1XSSC/0.1% SDS, 65 °C, and exposed overnight on Kodak XAR-2 film.

Semi-Quantitative RT-PCR Detection

30 The expression pattern of ALK-7 was also investigated using a PCR technique, RNA was isolated from a variety of human cell lines, fresh frozen tissues, and primary tumors as

detailed above. Single stranded cDNA was synthesized from 10 ug of each RNA as described above using the Superscript Preamplification System (GibcoBRL) These single strand templates were then used in a 35 cycle PCR reaction with two human
5 ALK-7-specific oligonucleotides:

ALK-7a: 5'-AACTTTGGCTGGTATCTGAATATC-3' (SEQ ID NO:69), and
ALK-7b: 5'-CCTTGTGTACCAACAATCTCCATA-3' (SEQ ID NO:70).

Reaction products were electrophoresed on 2% agarose gels,
10 stained with ethidium bromide and photographed on a UV light box. The relative intensity of the -150-bp ALK-7-specific bands were estimated for each sample. A similar pair of oligonucleotides was designed for detection of rat ALK-7:

15 4076: 5'-CTCCAGAGATGAGAGATCTTGG-3' (SEQ ID NO:71), and
4077: 5'-TTCCAGCCACGGTCACTATGTT-3' (SEQ ID NO:72),
encompassing a -210 bp region of the rat gene.

Results

20 ALK-7 mRNA transcript was not detectable by Northern analysis from multiple human tissue sources, suggesting its expression is highly restricted. Using a more sensitive PCR-based detection, ALK-7 was found to be expressed in human substantia nigra, anterior pituitary, and Calu-6 lung carcinoma
25 cell line (see below). Weak expression was found in several other locations including whole brain, cerebellum, and prostate. Multiple other normal human tissues and tumor cell lines showed no detectable ALK-7 expression.

HUMAN ALK-7 RNA EXPRESSION ANALYSIS

	Medium (++)	Negative
	Substantia Nigra	IMR-32 (neuroblastoma)
5	Anterior Pituitary	SY5Y (neuroblastoma)
	Calu-6 (Lung Ca)	SK-N-SH (neuroblastoma)
		SWI763 (astrocytoma)
		SW1388 (astrocytoma)
	<u>Weak (+)</u>	U-138 (glioblastoma)
10		U87MG (glioblastoma)
	Brain	Menirigiomas (1° tumors)
	Posterior Pituitary	SKOV-3 (ovarian Ca)
	Cerebellum	ASPC (pancreas Ca)
	Ovary	CAPAN-1 (pancreas Ca)
15	Prostate	HS766T (pancreas Ca)
	Fetal Intestine	PANC (pancreas Ca)
	Duodenum	HOS (osteosarcoma)
	T48 (colon Ca)	KHOS (osteosarcoma)
20		HTB227 (breast Ca)
		HTB131 (breast Ca)
		LS123 (colon Ca)
		LS147T (colon Ca)
		SkCO4 (colon Ca)
25		SW11E (colon Ca)
		HTC15 (colon Ca)
		SW403 (colon Ca)
		HT29 (colon Ca)
		SW627 (colon Ca)
		SW948 (colon Ca)
30		HUVEC (h. endothelial)
		Fibroblasts (Primary)
		Pancreas
		Testis
		Thymus
35		Liver
		Heart
		Placenta
		Lung
		Skel. Muscle
40		Kidney
		Spleen
		Ovary
		Colon
45		Leukocytes

In situ EXPRESSION PROFILE of RAT ALK-7

The neuronal expression of ALK-7 was assessed by in situ analysis in sagittal and coronal sections from neonatal and

adult rat brains using a fragment of the extracellular domain of rat ALK-7 as a probe. This region was selected because its dissimilarity with the related ALK-4 and ALK-5. Other groups have performed in situ with the catalytic domain of rat ALK-7 demonstrating specific expression in neuronal tissues (cerebellum, hippocampus, and brainstem nuclei), kidney, testis, lung, dorsolateral and anterior prostate, and adipose tissue. However, the probe used in these studies contained an ALK-7 catalytic domain which may cross-react with the related ALK-4 and ALK-5 (77% nucleotide sequence identity with stretches of 27/29 and 25/26 bp identity to rat ALK-7) and thereby broaden the expression profile. Using a more selective ALK-7 probe our analysis revealed the more restricted expression. In sagittal sections, a moderate strength granular band was visible in the CA2 and CA3 regions of the hippocampus, dentate gyrus, olfactory tubercle, dorsal outer layer of the cortex, and in a band crossing the frontal cortex area 2 from the exterior to the corpus callosum. A moderate signal was detected in the caudate putamen and thalamic nuclei. In addition, signals of moderate strength were detected in the region of the magnocellular nucleus of the lateral hypothalamus and the medial tuberal nucleus. A similar signal was observed in the region of the cuneiform nucleus on the anterior border of the cerebellum. The cerebellum was devoid of hybridizing ALK-7.

Coronal sections support the finding of expression in the CA2, CA3 region of the hippocampus, dentate gyrus, caudate putamen, and in the region underlying the exterior of the cortex. In addition, a signal of moderate strength was detected in the dorsomedial part of the ventromedial hypothalamic nucleus. A dispersed nuclei signal of lesser strength was detected in the area of the amygdalopiriform transition.

Example 24: ALK-7-Specific Antibodies

ALK-7-specific immunoreagents were raised in rabbits against KLH-conjugated synthetic peptide YRKKKRPNVEEPL (SEQ ID NO:76) from the juxtamembrane portion of the cytoplasmic domain of ALK-7. This region is unique to ALK-7 compared to other type I STK receptors, thereby allowing for the generation of ALK-7 specific antisera. The N-terminal extracellular domain of ALK-7 expressed as a GST-fusion was also used as an immunogen to raise polyclonal antibodies in rabbits and to generate monoclonal antibodies in mice using the techniques described above. These antibodies were used to localize expression of the endogenous and recombinant protein as describe below.

Example 25: Recombinant Alk-7 Expression

The following example describes the construction of vectors for transient and stable expression in mammalian cells. Expression constructs were generated to make wild type ALK-7 as well as a signaling incompetent ALK-7 (ALK-7DN) and a constitutively activated ALK-7 (ALK-7TA).

Materials and MethodsConstruction of Vectors

Expression constructs were generated by PCR-assisted mutagenesis in which the entire coding domain of ALK-7 was tagged at its carboxy-terminal ends with the hemophilus influenza hemagglutinin (HA) epitope YPYDVPDYAS (SEQ ID NO:77) (Pati, Gene 114:285, 1992). This constructs were introduced into two mammalian expression vectors: pAdRSVOES-, a modified adenovirus vector for the generation of virus producing recombinant protein, and pRK5 for transient expression analysis.

Recombinant adenoviruses were generated by in vivo ligation as follows.

The transfer vector used Contains the following DNA sequences in order: The left terminal region of adenovirus type 5 encoding the packaging sequences (adenovirus type 5 nucleotides 1-454); the Rous Sarcoma Virus long terminal repeat promoter and the SV40 polyA region, isolated as an expression cassette from the plasmid pREP (Invitrogen Corporation); nucleotides 3320-5790 of the type 5 adenoviral genome; and the ori and beta-lactamase genes derived from the *E. coli* plasmid pBluescript. Two additional forms of the plasmid were generated. The first, pAdRSVlacZ, was prepared by the insertion of a double stranded synthetic oligonucleotide into the BamHI restriction site between the RSV promotor and the SV40 polyA sequence with the following nucleotide sequence (upper strand shown): 5'

CTTCGAAAGCTTGAAATCGGTACCATCGATTCTAGAGTTAACTTCGAA. (SEQ ID NO: 73) The *E. coli* lacZ gene was excised from the expression plasmid pCMVb (Clontech, Inc.) with the enzyme Not I and inserted into the Not I site between the promoter and the polyA sequence. This generated a plasmid that expressed the lacZ gene, and had two BstBI restriction sites between the lacZ gene and the polyA region. The second plasmid (pAdRSVOES-) was generated by inserting a double stranded synthetic oligonucleotide into the same region as above. Its nucleotide sequence was the following: 5'

CTCTAGAACGCGTTAAGGCGCGCCAATATCGATGAATTCTTCGAAGC. (SEQ ID NO:74) This plasmid allowed the introduction of exogenous cDNAs into the plasmid for expression purposes.

The viral DNA used for generation of recombinant viruses was derived from a virus (AdlacZBstBI) in which the left end of the adenovirus genome has been replaced by the homologous region of pAdRSVlacZ. To achieve this, DNA ,was isolated from

the Ad5 dl327 strain of adenovirus (Jones and Shenk, Cell, 1978) (deleted in the E3 region), cleaved with ClaI enzyme, and cotransfected into the HEK2934 cell line via calcium phosphate coprecipitation with the pAdRSVlacZ plasmid. Recombinant adenovirus plaques resulting from this transfection were screened for the ability to express the lacZ gene by histochemical staining with X-Gal. The resulting recombinant adenovirus, AdlacZBstBI, provided the backbone for additional adenovirus constructs, allowing a screen for recombinant plaques based on the presence or absence of lacZ activity in that further recombination would replace the lacZ gene with the cotransfected cDNA. To achieve this, the transfer vector construct is linearized by digestion with BstBI, and cotransfected with AdlacZBstBI DNA which has also been cleaved with BstBI. Typically, 5 mg of transfer vector plasmid DNA are corecipated with 2 mg of viral DNA for the transfection; in vivo ligation of viral DNA and linearized transfer vector produces a novel recombinant virus directing expression of the new transgene.

A signaling incompetent ALK-7 construct was also made in both vectors pAdRSVOES- and pRK5 by insertion of an HA-tag at aa 230 in the ALK-7 coding region just after catalytic domain II. Truncation of other Type I STKRs in an analogous location has functioned in a dominant negative manner. This construct was called ALK-7DN. A constitutively active form of ALK-7 was generated by a Thr to Asp mutation at amino acid 194 just upstream of the catalytic domain I GXGXXG motif. In other Type I STKRs, this residue undergoes ligand-dependent transphosphorylation by the associated Type II STKR, resulting receptor activation and initiation of a signaling cascade. A similar mutation in other Type I STKR's results in a ligand-independent, constitutively activated receptor. This construct was called ALK-7TD.

Generation Of Recombinant ALK-7 - Adenovirus

Early passage HEK293 cells (Graham, et al., J. Gen. Virol. 36:59, 1977) were maintained in Dulbecco's modified
5 Eagles medium + 10% calf serum. HEK293 monolayers were transfected with the ALK-7-encoding transfer vectors and cultured from five to seven days to allow plaques to appear. The monolayers were then stained with 25 mg/mL 5-bromo-4-chloro-7-indolyl- β -D-galactopyranoside for several hours to
10 identify non-recombinant (blue-stained) plaques. Putative recombinant plaques were screened for expression of the transgene by infection of HEK293 cultures followed by immunohistochemistry with the monoclonal antibody recognizing the HA epitope. Viruses which were positive for transgene
15 protein expression were picked and subjected to several rounds of plaque purification prior to amplification and purification on cesium chloride gradients. Banded viruses were diluted five-fold with dilution buffer (Curiel et al., Proc. Natl. Acad. Sci., USA 88:8850-8854, 1991) and stored at -80 °C.
20 Approximate titers of the virus preparations were determined immunohistochemically on HEK293 cultures. The following viruses were generated: AdRSVALK-7-HA; AdRSVALK-7-DN; and AdRSVALK-7-TD.

25 Transient Expression

The pRK5 expression plasmids (10 μ g DNA/100 mm plate) containing the KA-tagged ALK-7, the ALK-7DN, and ALK-7TD constructs were introduced into COS and 293 cells with lipofectamine (Gibco BRL). After 72 hours, the cells were
30 harvested in 0.5 ml solubilization buffer (20 mM HEPES pH 7.35, 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM MgCl₂, 1 mM EGTA, 2 mM phenylmethylsulfonyl fluoride, 1 μ g/mL aprotinin).

Sample aliquots were resolved by SDS polyacrylamide gel electrophoresis (PAGE) on 15% acrylamide/0.5% bis-acrylamide gels and electroplicretically transferred to nitrocellulose. Non-specific binding was blocked by preincubating blots in Blotto (phosphate buffered saline containing 5% w/v non-fat dried milk and 0.2% v/v nonidet P-40 (Sigma)), and recombinant protein was detected using a murine Mab to the HA decapeptide tag. Alternatively, recombinant protein can be detected using various ALK-7-specific antisera.

Expression In Neuronal Cells

The recombinant ALK-7 protein described above were expressed in PC12 cells and primary rat neuronal cultures by adenovirus mediated infection. These cells will allow further investigation into ALK-7 function. Recombinant protein expression was confirmed by immunostaining with an anti-HA antibody.

PC12 cultures (Greene, et al., Methods Enzymol. 147:207, 1987) were maintained in RPMI medium containing 10% horse serum and 5% fetal calf serum. Four differentiation experiments the medium was changed to RPMI containing 1X N2 supplement and 0.1% BSA, and the cells were grown on a collagen I substrate. For PC12 cell survival, the cells were grown in RPMI containing 0.1% BSA. All cultures also contained 1X penicillin/streptomycin. For adenoviral infections, PC12 cells were incubated overnight with recombinant viruses at a multiplicity of infection (MOI) between 1 and 10. The cells were then washed and replated either into differentiation or survival conditions for two days. Nerve Growth Factor (50 ng/mL) served as a positive control. For differentiation, the cultures were fixed with 2% paraformaldehyde and the percentage of cells bearing processes longer than 1 cell diameter was determined. For survival, the cultures were incubated with 0.05% MTT for

1.5 hours to stain living cells, and the relative number of cells surviving in each condition was determined.

Sympathetic and sensory neurons were isolated as described (Hawrot and Patterson, Methods Enzymol. 53:574, 1979; Fields et al., Cell 14:43, 1978) and cultured in a defined medium (Hawrot and Patterson, *supra*). Sympathetic neurons were isolated from superior cervical ganglia dissected from E20 - E21 rat fetuses, while dorsal root ganglion sensory neurons were obtained from E16 - E18 rats. The ganglia were treated with 0.25% trypsin for 10 minutes, washed, and triturated to obtain a single cell suspension. Sensory neurons were preplated for 1 hour on tissue culture plastic to deplete adherent cells. Dopaminergic neurons were isolated as described (Shimoda, et al., Brain Research 586:319-331, 1992) and cultured in Neurobasal medium, supplemented with B27 supplements (Life Technologies). Neurons were infected with adenoviruses for two hours on collagen I-coated tissue culture plastic (supplemented with NGF for sensory and sympathetic neurons), and the cells were then washed and allowed to recover for two to four additional hours (with NGF if appropriate). After the recovery period, the cells were washed extensively to remove the growth factor, and plated onto polylysine-laminin coated chamber slides. The addition of NGF at 50 ng/mL served as a positive control for survival of sensory and sympathetic neurons. After an additional two days to three days, the sensory and sympathetic cultures were stained with calcein AM (1 mg/mL) for 45 minutes, mounted and examined by immunofluorescence. Generally, five disperse fields representing 7% of the well were photographed and the number of surviving neurons quantitated. To determine dopaminergic neuron survival, the cultures were fixed and the number of tyrosine hydroxylase positive neurons was determined.

Results

Recombinant ALK-7 protein expressed in COS cells migrated with apparent Mr of 52kD-63kD, consistent with its predicted molecular weight of 54kD based on its primary amino acid sequence and the presence of multiple glycosylation sites. The ALK-7TD constitutive active form produced proteins indistinguishable from the wild type construct on SDS-PAGE. The ALK-7DN construct expressed proteins of Mr 23.5 kd, 28 kd and 32 kd consistent with the presence of varying amounts of glycosylation on this truncated receptor. This analysis confirms the recombinant protein can be stably produced in mammalian cells.

One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The molecular complexes and the methods, procedures, treatments, molecules, specific compounds described herein are presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art which are encompassed within the spirit of the invention are defined by the scope of the claims.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention.

All patents and publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the

terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group. For example, if X is described as selected from the group consisting of bromine, chlorine, and iodine, claims for X being bromine and claims for X being bromine and chlorine are fully described.

In view of the degeneracy of the genetic code, other combinations of nucleic acids also encode the claimed peptides and proteins of the invention. For example, all four nucleic acid sequences GCT, GCC, GCA, and GCG encode the amino acid alanine. Therefore, if for an amino acid there exists an average of three codons, a polypeptide of 100 amino acids in length will, on average, be encoded by 3^{100} , or 5×10^{47} , nucleic acid sequences. It is understood by those skilled in the art that, with, Thus, a nucleic acid sequence can be modified to form a second nucleic acid sequence, encoding the same

polypeptide as encoded by the first second nucleic acid sequences, using routine procedures and without undue experimentation. Thus, all possible nucleic acids that encode the claimed peptides and proteins are also fully described herein, as if all were written out in full taking into account the codon usage, especially that preferred in humans.

Furthermore, changes in the amino acid sequences of polypeptides, or in the corresponding nucleic acid sequence encoding such polypeptide, may be designed or selected to take place in an area of the sequence where the significant activity of the polypeptide remains unchanged. For example, an amino acid change may take place within a β -turn, away from the active site of the polypeptide. Also changes such as deletions (e.g. removal of a segment of the polypeptide, or in the corresponding nucleic acid sequence encoding such polypeptide, which does not affect the active site) and additions (e.g. addition of more peptides to the polypeptide sequence without affecting the function of the active site, such as the formation of GST-fusion proteins, or additions in the corresponding nucleic acid sequence encoding such polypeptide without affecting the function of the active site) are also within the scope of the present invention. Such changes to the polypeptides can be performed by those with ordinary skill in the art using routine procedures and without undue experimentation. Thus, all possible nucleic and/or amino acid sequences that can readily be determined not to affect a significant activity of the peptide or protein of the invention are also fully described herein.

Other embodiments are within the following claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: SUGEN, INC.
351 Galveston Drive
Redwood City, CA 94063
U.S.A.

10 (ii) TITLE OF INVENTION: DIAGNOSIS AND TREATMENT OF
TYROSINE PHOSPHATASE-RELATED
DISORDERS AND RELATED METHODS

15 (iii) NUMBER OF SEQUENCES: 76

(iv) CORRESPONDENCE ADDRESS:

20 (A) ADDRESSEE: Lyon & Lyon
(B) STREET: 633 West Fifth Street
Suite 4700
(C) CITY: Los Angeles
(D) STATE: California
25 (E) COUNTRY: U.S.A.
(F) ZIP: 90071-2066

(v) COMPUTER READABLE FORM:

30 (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
storage
(B) COMPUTER: IBM Compatible
(C) OPERATING SYSTEM: IBM P.C. DOS 5.0
35 (D) SOFTWARE: FastSEQ for Windows 2.0

(vi) CURRENT APPLICATION DATA:

40 (A) APPLICATION NUMBER: To be assigned
(B) FILING DATE: Herewith
(C) CLASSIFICATION:

45 (vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 60/044,428
(B) FILING DATE: April 28, 1997

50 (A) APPLICATION NUMBER: US 60/047,222
(B) FILING DATE: May 20, 1997

(A) APPLICATION NUMBER: US 60/049,477
(B) FILING DATE: June 12, 1997

55 (A) APPLICATION NUMBER: US 60/049,756
(B) FILING DATE: June 12, 1997

(A) APPLICATION NUMBER: US 60/049,914
(B) FILING DATE: June 18, 1997

60 (A) APPLICATION NUMBER: US 60/063,595
(B) FILING DATE: October 23, 1997

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Warburg, Richard J.
(B) REGISTRATION NUMBER: 32,327
(C) REFERENCE/DOCKET NUMBER: 233/032-PCT

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (213) 489-1600
(B) TELEFAX: (213) 955-0440
(C) TELEX: 67-3510

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 3580 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CCCCGGGTGCC	CTCCCTCAAC	CTACTTATAG	ACTATTTTTC	TTGCTCTGCA	GCATGGACCA	60
AAGAGAAATT	CTGCAGAAGT	TCCTGGATGA	GGCCCAAAGC	AAGAAAATTA	CTAAAGAGGA	120
GTTTGCCAAT	GAATTTCTGA	AGCTGAAAAG	GCAATCTACC	AAGTACAAGG	CAGACAAAAC	180
CTATCCTACA	ACTGTGGCTG	AGAAGCCCAA	GAATATCAAG	AAAAACAGAT	ATAAGGATAT	240
TTTGCCCTAT	GATTATAGCC	GGGTAGAAGT	ATCCCTGATA	ACCTCTGATG	AGGATTCCAG	300
CTACATCAAT	GCCAACTTCA	TTAAGGGAGT	TTATGGACCC	AAGGCTTATA	TTGCCACCCA	360
GGGTCCTTTA	TCTACAACCC	TCCTGGACTT	CTGGAGGATG	ATTTGGGAAT	ATAGTGTCTT	420
TATCATTGTT	ATGGCATGCA	TGGAGTATGA	AATGGGAAAG	AAAAAGTGTG	AGCGCTACTG	480
GGCTGAGCCA	GGAGAGATGC	AGCTGGAATT	TGGCCCTTTC	TCTGTATCCT	GTGAAGCTGA	540
AAAAAGGAAA	TCTGATTATA	TAATCAGGAC	TCTAAAAGTT	AAGTTCAATA	GTGAAACTCG	600
AACTATCTAC	CAGTTTCATT	ACAAGAATTG	GCCAGACCAT	GATGTACCTT	CATCTATAGA	660
CCCTATTCTT	GAGCTCATCT	GGGATGTACG	TTGTTACCAA	GAGGATGACA	GTGTTCCCAT	720
ATGCATTTC	TGCAGTGCTG	GCTGTGGAAG	GACTGGTGTT	ATTTGTGCTA	TTGATTATAC	780
ATGGATGTTG	CTAAAAGATG	GGATAATTCC	TGAGAACTTC	AGTGTTTTCA	GTTTGATCCG	840
GGAAATGCGG	ACACAGAGGC	CTTCATTAGT	TCAAACGCAG	GAACAATATG	AACTGGTCTA	900
CAATGCTGTA	TTAGAAGATA	TTAAGAGACA	GATGGATGTT	ATCAGAGATA	AACATTCTGG	960
AACAGAGAGT	CAAGCAAAGC	ATTGTATTCC	TGAGAAAAAT	CACACTCTCC	AAGCAGACTC	1020
TTATTCTCCT	AATTTACCAA	AAAGTACCAC	AAAAGCAGCA	AAAATGATGA	ACCAACAAAG	1080
GACAAAAATG	GAAATCAAAG	AATCTTCTTC	CTTTGACTTT	AGGACTTCTG	AAATAAGTGC	1140
AAAAGAAGAG	CTAGTTTTGC	ACCCTGCTAA	ATCAAGCACT	TCTTTTGACT	TTCTGGAGCT	1200
AAATTACAGT	TTTGACAAAA	ATGCTGACAC	AACCATGAAA	TGGCAGACAA	AGGCATTTCC	1260
AATAGTTGGG	GAGCCTCTTC	AGAAGCATCA	AAGTTTGGAT	TTGGGCTCTC	TTTTGTTTGA	1320
GGGATGTTCT	AATTCTAAAC	CTGTAATATG	AGCAGGAAGA	TATTTTAATT	CAAAGGTGCC	1380
AATAACACGG	ACCAAATCAA	CTCCTTTTGA	ATTGATACAG	CAGAGAGAAA	CCAAGGAGGT	1440
GGACAGCAAG	GAAAACTTTT	CTTATTTGGA	ATCTCAACCA	CATGATTCTT	GTTTTGTAGA	1500
GATGCAGGCT	CAAAAAGTAA	TGCATGTTTC	TTCAGCAGAA	CTGAATTATT	CACTGCCATA	1560
TGACTCTAAA	CACCAAATAC	GTAATGCCTC	TAATGTAAAG	CACCATGACT	CTAGTGCTCT	1620
TGGTGTATAT	TCTTACATAC	CTTGTAGTGA	AAATCCTTAT	TTTTTCATCAT	GGCCTCCAAG	1680
TGGTACCAGT	TCTAAGATGT	CTCTTGATTT	ACCTGAGAAG	CAAGATGGAA	CTGTTTTTCC	1740
TTCTTCTCTG	TTGCCAACAT	CCTCTACATC	CCTCTTCTCT	TATTACAATT	CACATGATTC	1800
TTTATCACTG	AATTCTCCAA	CCAATATTTC	CTCATATTG	AACCAGGAGT	CAGCTGTACT	1860
AGCAACTGCT	CCAAGGATAG	ATGATGAAAT	CCCCCTCCA	CTTCCTGTAC	GGACACCTGA	1920
ATCATTTATT	GTGTTGAGG	AAGCTGGAGA	ATTCTCACCA	AATGTTCCCA	AATCCTTATC	1980
CTCAGCTGTG	AAGCTAAAAA	TTGGAACATC	ACTGGAATGG	GGTGGAAACAT	CTGAACCAAA	2040
GAAATTTGAT	GACTCTGTGA	TACTTAGACC	AAGCAAGAGT	GTAAGTCTCC	GAAGTCCTAA	2100
ATCAGAACTA	CATCAAGATC	GTTCTTCTCC	CCCACCTCTC	CTCCAGAGAA	GAACCTTAGA	2160
GTCCTTCTTT	CTTGCCGATG	AAGATTGTAT	GCAGGCCCAA	TCTATAGAAA	CATATTCTAC	2220
TAGCTATCCT	GACACCATGG	AAAATTCAAC	ATCTTCAAAA	CAGACACTGA	AGACTCCTGG	2280

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	AAAAAGTTTC	ACAAGGAGTA	AGAGTTTGAA	AATTTTGC GA	AACATGAAAA	AGAGTATCTG	2340
	TAATTCTTGC	CCACCAACA	AGCCTGCAGA	ATCTGTTCAG	TCAAATAACT	CCAGCTCATT	2400
	TCTGAATTTT	GGTTTGTCAA	ACCGTTTTTC	AAAACCCAAA	GGACCAAGGA	ATCCACCACC	2460
5	AACCTGGAAT	ATTTAATAAA	ACTCCAGATT	TATAATAATA	TGGGCTGCAA	GTACACCTGC	2520
	AAATAAAACT	ACTAGAATAC	TGCTAGTTAA	AATAAGTGCT	CTATATGCAT	AATATCAAAT	2580
	ATGAAGATAT	GCTAATGTGT	TAATAGCTTT	TAAAAGAAAA	GCAAATGCC	AATAAGTGCC	2640
	AGTTTTGCAT	TTTCATATCA	TTTGCATGA	GTTGAAAACT	GCAAATAAAA	GTTTGTCACT	2700
	TGAGCTTATG	TACAGAATGC	TATATGAGAA	ACACTTTTAG	AATGGATTTA	TTTTTCATTT	2760
	TTGCCAGTTA	TTTTTATTTT	CTTTTACTTT	TTTACATAAA	CATAAACTTC	AAAAGGTTTG	2820
10	TAAGATTTGG	ATCTCAACTA	ATTTCTACAT	TGCCAGAATA	TACTATAAAA	AGTTAAAAAA	2880
	AAACTTACTT	TGTGGGTTGC	AATACAAACT	GCTCTTGACA	ATGACTATTC	CCTGACAGTT	2940
	ATTTTTGCCT	AAATGGAGTA	TACCTTGTAA	ATCTTCCCAA	ATGTTGTGGA	AACTGGAAT	3000
	ATTAAGAAAA	TGAGAAATTA	TATTTATTAG	AATAAAATGT	GCAAATAATG	ACAATTATTT	3060
	GAATGTAACA	AGGAATTCAA	CTGAAATCCT	GATAAGTTTT	AACCAAAGTC	ATTAAATTAC	3120
15	CAATTCTAGA	AAAGTAATCA	ATGAAATATA	ATAGCTATCT	TTTGGTAGCA	AAAGATATAA	3180
	ATTGTATATG	TTTATACAGG	ATCTTTCAGA	TCATGTGCAA	TTTTTATCTA	ACCAATCAGA	3240
	AATACTAGTT	TAAAATGAAT	TTCTATATGA	ATATGGATCT	GCCATAAGAA	AATCTAGTTC	3300
	AACCTAATT	TTATGTAGTA	AATAAATTGG	CAGGTAATTG	TTTTTACAAA	GAATCCACCT	3360
	GACTTCCCTT	AATGCATTA	AAATATTTT	ATTTAAATAA	CTTTATTTAT	AACTTTTAGA	3420
20	AACATGTAGT	ATTGTTTAAA	CATCATTTGT	TCTTCAGTAT	TTTTCATTTG	GAAGTCCAAT	3480
	AGGGCAAATT	GAATGAAGTA	TTATTATCTG	TCTCTTGTAG	TACAATGTAT	CCAACAGACA	3540
	CTCAATAAAC	TTTTTGGTTG	TTAAAAAAA	AAAAAAA			3580

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

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(A)	LENGTH:	1548 base pairs
(B)	TYPE:	nucleic acid
(C)	STRANDEDNESS:	single
(D)	TOPOLOGY:	linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

	GCTCGCGGGC	TCCCATGGCC	CTCGGGCCCA	GCGTGGTGAC	CCCGGGGGAT	GGAGCCGTTC	60
	CTCAGGAGGC	GGCTGGCCTT	CCTGTCCTTC	TTCTGGGACA	AGATCTGGCC	GGCGGGCGGC	120
	GAGCCGGACC	ATGGCACCCC	CGGGTCCCTG	GACCCCAACA	CTGACCCAGT	GCCCACGCTC	180
40	CCCGCCGAGC	CTTGCGAGCC	CTTCCCTCAG	CTCTTCCTTG	CGCTCTATGA	CTTCACGGCG	240
	CGGTGTGGCG	GGGAGCTGAG	TGTCCGCCGC	GGGGACAGGC	TCTGTGCCCT	CGAAGAGGGG	300
	GGCGGCTACA	TCTTCGCACG	CAGGCTTTCG	GGCCAGCCCA	GCGCCGGGCT	CGTGCCCATC	360
	ACCCACGTGG	CCAAGGCTTC	TCCTGAGACG	CTCTCAGACC	AACCCTGGTA	CTTTAGCGGG	420
	GTCAGTCGGA	CCCAGGCACA	GCAGCTGCTC	CTCTCCCCAC	CCAACGAACC	AGGGGCCTTC	480
45	CTCATCCGGC	CCAGCGAGAG	CAGCCTCGGG	GGCTACTCAC	TGTCAGTCCG	GGCCCAGGCC	540
	AAGGTCTGCC	ACTACCGGGT	CTCCATGGCA	GCTGATGGCA	GCCTCTACCT	GCAGAAGGGA	600
	CGGCTCTTTC	CCGGCCTGGA	GGAGCTGCTC	ACCTACTACA	AGGCCAACTG	GAAGCTGATC	660
	CAGAACCCCC	TGCTGCAGCC	CTGCATGCCC	CAGAAGGCCC	CGAGGCAGGA	CGTGTGGGAG	720
	CGGCCACACT	CCGAATTTCG	CCTTGGGAGG	AAGCTGGGTG	AAGGCTACTT	TGGGGAGGTG	780
50	TGGGAAGGCC	TGTGGCTGGG	CTCCCTGCCC	GTGGCGATCA	AGGTCATCAA	GTCAGCCAAC	840
	ATGAAGCTCA	CTGACCTCGC	CAAGGAGATC	CAGACACTGA	AGGGCCTGCG	GCACGAGCGG	900
	CTCATCCGGC	TGCACGCAGT	GTGCTCGGGC	GGGGAGCCTG	TGTACATAGT	CACGGAATCT	960
	ATGCGCAAGG	GGAACCTGCA	GGCCTTCCTG	GGCACCCCGG	AGGGCCGGGC	CCTGCGTCTG	1020
	CCGCCACTCC	TGGGCTTTGC	CTGCCAGGTG	GCTGAGGGCA	TGAGCTACCT	GGAGGAGCAG	1080
55	CGCGTTGTGC	ACCGGGACTT	GGCCGCCCGG	AACGTGCTCG	TGGACGACGG	CCTGGCCTGC	1140
	AAGGTGGCTG	ACTTCGGCCT	GGCCCGGCTG	CTCAAGGACG	ACATCTACTC	CCCAGCAGC	1200
	AGTCCAAGA	TCCCGGTCAA	GTGGACAGCG	CCTGAGGCGG	CCAATTATCG	TGTCTTCTCC	1260
	CAGAAGTCAG	ACGCTGTGTC	CTTCGGCGTG	CTGTGTCACG	AGGTTTTCAC	CTATGGCCAG	1320
	TGTCCCTATG	AAGGGATGAC	CAACCACGAG	ACGCTGCAGC	AGATCATGCG	AGGGTACCGG	1380
60	CTGCCGCGCC	CGGCTGCCTG	CCCGGCGGAG	GTCTACGTGC	TCATGCTGGA	GTGCTGGAGG	1440
	AGCAGCCCCG	AGGAACGGCC	CTCCTTTGCC	ACGCTGCGGG	AGAAGCTGCA	CGCCATCCAC	1500
	AGATGCCACC	CCTGAGTCTT	CACGTGACCC	AACGCTCTGG	GCTCCAGC		1548

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(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1785 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

15 GGTATGTCT GACTCACTGC ACTGGAGTTT GGCAAAAGCA TCTCAGAAGT GGTGTGCTT 60
 TTTTGAATGA AATGATCAAT GGAGTGCTCC AGTTGTATGC TGGCCTCTGG ATACTAACTA 120
 GACCTGCCTG ACTCCAGGAA CTAAGGCTCA GTATCTGCAG AAGCTTTTTG CCCATCTCAT 180
 TCCGGCTATG GGGACAACAT GTCTTCACCC AGGAAGGTTA GAGGAAAAAC TGGAAGAGAT 240
 AATGATGAAG AGGAGGGTAA TTCAGGTAAC CTGAATCTCC GCAACTCTTT GCCTTCATCG 300
 20 AGTCAGAAAA TGACGCCTAC GAAGCCGATT TTTGGGAATA AAATGAATTC AGAGAATGTA 360
 AAACCTCCCT ATCACCTGTC ATTCTCAGAT AAGTATGAGC TTGTTTACCC AGAGCCTTTG 420
 GAAAGTGACA CTGATGAGAC TGTGTGGGAT GTCAGTGACC GGTCTCTCAG AAACAGGTGG 480
 AACAGTATGG ATTCAGAGAC TGCAGGGCCG TCAAAGACTG TCTCCCCAGT GCTTCTGCTG 540
 AGTAGTAGGC TCTCAAAGGA CACTGAAACA TCTGTCTCTG AAAAGGAGCT AACTCAGTTG 600
 GCTCAGATTG GACCATTAAT ATTCAACAGT TCTGCACGGT CTGCTATGCG GGATTGTTTG 660
 25 AACACGCTTC AGAAAAAAGA AGAACTTGAT ATCATCCGTG AGTTTTTGA GTTAGAACAA 720
 ATGACTCTGC CTGATGACTT CAATTCTGGG AATACACTAC AGAACAGAGA TAAGAACAGA 780
 TACCGAGATA TTCTTCATA TGATTCAACA CGTGTTCCTC TTGGAAAAAA CAAGGACTAC 840
 ATCAACGCTA GTTATATTAG AATAGTAAAT CATGAAGAAG AGTATTTTTA TATTGCCACT 900
 CAAGGACCAT TGCCAGAAAC TATAGAAGAC TTTTGGCAA TGGTCTGGA AAATAATTGT 960
 30 AATGTTATTG CTATGATAAC CAGAGAGATA GAATGTGGAG TTATCAAGTG TTACAGTTAC 1020
 TGGCCCATTT CTCTGAAGGA GCCTTTGGAA TTCGAACACT TTAGTGTCTT TCTGGAGACC 1080
 TTTTCATGTAA CTCAATATTT CACCGTTCGA GTATTTTCTG TTGTGAAGAA GTCCACAGGA 1140
 AAGAGCCAAT GTGTAAACA CTTGCAGTTC ACCAAGTGGC CAGACCATGG CACTCCTGCC 1200
 TCAGCAGATT TTTTCATAA ATATGTCCGT TATGTGAGGA AGAGCCACAT TACAGGACCC 1260
 35 CTCCTTGTTT ACTGCAGTGC TGGTGTAGCG CGAACAGGGG TGTTTCATATG TGTGGATGTT 1320
 GTGTCTCTG CCATCGAGAA GAACTACTCT TTTGACATTA TGAACATAGT GACCCAGATG 1380
 AGAAAGCAGC GCTGTGGCAT GATTCAAACC AAGGAGCAGT ACCAGTTTTG TTATGAAATT 1440
 GTGCTTGAAG TTCTTCAGAA CCTTCTGGCT TTGTATTAAG AGAGACTTCT GCGCCTGTCC 1500
 CTCGAGTTA CCGAGCAGCT TGGAGCCTGA GCCGTCTGCA AGCGTCTGCG GGCCGTGCAG 1560
 40 TCTGCCTTCT GATTTTTCTC TCTGAAAGTC CCTGAAGGTA GCACTACTGG GCACAGAGTG 1620
 AACTGTTTCC ACTTGATCTT TCTGAACAAG AGCAAAATAC CCTCCATGCC TTCTACGGAA 1680
 ACGGAAGTTG CATGAACAA CCTCCGCTTG GCTGTCTGGT TTGTGGTATT ACAGAGCTTA 1740
 ATAAAGACT TAGATGTGAA AAAAAAAAAA AAAAAAAAAA AAAAA 1785

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1896 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

60 GGTATGTCT GACTCACTGC ACTGGAGTTT GGCAAAAGCA TCTCAGAAGT GGTGTGCTT 60
 TTTTGAATGA AATGATCAAT GGAGTGCTCC AGTTGTATGC TGGCCTCTGG ATACTAACTA 120
 GACCTGCCTG ACTCCAGGAA CTAAGGCTCA GTATCTGCAG AAGCTTTTTG CCCATCTCAT 180
 TCCGGCTATG GGGACAACAT GTCTTCACCC AGGAAGGTTA GAGGAAAAAC TGGAAGAGAT 240
 AATGATGAAG AGGAGGGTAA TTCAGGTAAC CTGAATCTCC GCAACTCTTT GCCTTCATCG 300
 AGTCAGAAAA TGACGCCTAC GAAGCCGTTA CAAAATAAAA ATCTCATGAA GTATGAAGAA 360
 65 CACTTAGATA TATTGATGGT GTTTTATTG ATAAAAACCA TATGGTATAA TGTCTTCAA 420
 TTATGGAAAG GCAAGCTTAT TTTTGGGAAT AAAATGAATT CAGAGAATGT AAAACCTCC 480

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	CATCACCTGT	CATTCTCAGA	TAAGTATGAG	CTTGTTTACC	CAGAGCCTTT	GGAAAGTGAC	540
	ACTGATGAGA	CTGTGTGGGA	TGTCAGTGAC	CGGTCTCTCA	GAAACAGGTG	GAACAGTATG	600
	GATTTCAGAGA	CTGCAGGGCC	GTCAAAGACT	GTCTCCCCAG	TGCTTCTCTGG	TAGTAGTAGG	660
5	CTCTCAAAGG	ACACTGAAAC	ATCTGTCTCT	GAAAAGGAGC	TAACCTAGTT	GGCTCAGATT	720
	CGACCATTAA	TATTTCAACAG	TTCTGCACGG	TCTGCTATGC	GGGATTGTTT	GAACACGCTT	780
	CAGAAAAAAG	AAGAACTTGA	TATCATCCGT	GAGTTTTTGG	AGTTAGAACA	AATGACTCTG	840
	CCTGATGACT	TCAATTCTGG	GAATACACTA	CAGAACAGAG	ATAAGAACAG	ATACCGAGAT	900
	ATTCTTCCAT	ATGATTCAAC	ACGTGTTCCCT	CTTGAAAAAA	ACAAGGACTA	CATCAACGCT	960
	AGTTATATTA	GAATAGTAAA	TCATGAAGAA	GAGTATTTTT	ATATTGCCAC	TCAAGGACCA	1020
10	TTGCCAGAAA	CTATAGAAGA	CTTTTGGCAA	ATGGTCTCTG	AAAAAATTG	TAATGTTATT	1080
	GCTATGATAA	CCAGAGAGAT	AGAATGTGGA	GTTATCAAGT	GTTACAGTTA	CTGGCCCAT	1140
	TCTCTGAAGG	AGCCTTTGGA	ATTCTGAACAC	TTTAGTGTCT	TTCTGGAGAC	CTTTCATGTA	1200
	ACTCAATATT	TCACCGTTTC	AGTATTTTCAG	ATTGTGAAGA	AGTCCACAGG	AAAGAGCCAA	1260
	TGTGTAAAAC	ACTTGCAGTT	CACCAAGTGG	CCAGACCATG	GCACTCCTGC	CTCAGCAGAT	1320
15	TTTTTCATAA	AATATGTCCG	TTATGTGAGG	AAGAGCCACA	TTACAGGACC	CCTCCTTGTT	1380
	CACTGTCAGT	CTGGTGTAGG	CCGAACAGGG	GTGTTTCATAT	GTGTGGATGT	TGTGTTCTCT	1440
	GCCATCGAGA	AGAACTACTC	TTTTGACATT	ATGAACATAG	TGACCCAGAT	GAGAAAGCAG	1500
	CGCTGTGGCA	TGATTCAAAC	CAAGGAGCAG	TACCACTTTT	GTTATGAAAT	TGTGCTTGAA	1560
	GTTCTTCAGA	ACCTTCTGGC	TTTGTATTAA	GAGAGACTTC	TGCGCCTGTC	CCTCGAGGTT	1620
20	ACCGAGCAGC	TTGGAGCCTG	AGCCGTGCTG	AAGCGTCTGT	GGGCCGTGCA	GTCTGCCCTC	1680
	TGATTTTTCT	CTCTGAAAGT	CCCTGAAGGT	AGCACTACTG	GGCAGAGAGT	GAAGTGTTC	1740
	CACTTGATCT	TTCTGAACAA	GAGCAAAATA	CCCTCCATGC	CTTCTACGGA	AACGGAAGTT	1800
	GCATGAAACA	ACCTCCGCTT	GGCTGTCTGG	TTGTGGGTAT	TACAGAGCTT	AATAAAAGAC	1860
25	TTAGATGTGA	AAAAAAAAAA	AAAAAAAAAA	AAAAAA			1896

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH:	1692 base pairs
(B) TYPE:	nucleic acid
(C) STRANDEDNESS:	single
(D) TOPOLOGY:	linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

	GGTTATGTCT	GACTCACTGC	ACTGGAGTTT	GGCAAAAGCA	TCTCAGAAAGT	GGTTGTGCTT	60
	TTTTGAATGA	AATGATCAAT	GGAGTGCTCC	AGTTGTATGC	TGGCCTCTGG	ATACTAATA	120
40	GACCTGCCTG	ACTCCAGGAA	CTAAGGCTCA	GTATCTGCAG	AAGCTTTTTG	CCCATCTCAT	180
	TCCGGCTATG	GGGACAACAT	GTCTTCACCC	AGGAAGGTTA	GAGGAAAAAC	TGGAAGAGAT	240
	AATGATGAAG	AGGAGGGTAA	TTCAGGTAAC	CTGAATCTCC	GCAACTCTTT	GCCTTCATCG	300
	AGTCAGAAAA	TGACGCCTAC	GAAGCCGATT	TTTGGGAATA	AAATGAATTC	AGAGAATGTA	360
	AAACCTCCC	ATCACCTGTC	ATTCTCAGAT	AAGTATGAGC	TTGTTTACCC	AGAGCCTTTG	420
45	GAAAGTGACA	CTGATGAGAC	TGTGTGGGAT	CAAGTGACC	GGTCTCTCAG	AAACAGGTGG	480
	AACAGTATGG	ATTCTAGAGAC	TGCAGGGCCG	TCAAAGACTG	TCTCCCCAGT	GCTTCTCTGGT	540
	AGTAGTAGGC	TCTCAAAGGA	CACTGAAACA	TCTGTCTCTG	AAAAGGAGCT	AACTCAGTTG	600
	GCTCAGATTC	GACCATTAAT	ATTCAACAGT	TCTGCACGGT	CTGCTATGCG	GGATTGTTTG	660
	AACACGCTTC	AGAAAAAAGA	AGAACTTGAT	ATCATCCGTG	AGTTTTTGGA	GTTAGAACAA	720
50	ATGACTCTGC	CTGATGACTT	CAATTCTGGG	AATACACTAC	AGAACAGAGA	TAAGAACAGA	780
	TACCGAGATA	TTCTTCCATA	TGATTCAACA	CGTGTTCTCT	TTGGAAAAAA	CAAGGACTAC	840
	ATCAACGCTA	GTTATATTAG	AATAGTAAAT	CATGAAGAAG	AGTATTTTTA	TATTGCCACT	900
	CAAGGACCAT	TGCCAGAAAC	TATAGAAGAC	TTTTTGGCAA	TGGTCTCGGA	AAATAATTGT	960
	AATGTTATTG	CTATGATAAC	CAGAGAGATA	GAATGTGGAG	TTATCAAGTG	TTACAGTTAC	1020
55	TGGCCCATTT	CTCTGAAGGA	GCCTTTGGAA	TTTGAACACT	TTAGTGTCTT	TCTGGAGACC	1080
	TTTCATGTAA	CTCAATATTT	CACCGTTCTGA	GTATTTTCAGA	TTGTGAAGAA	GTCCACAGGA	1140
	AAGAGCCAAT	GTGTAAAACA	CTTGCAGTTC	ACCAAGTGGC	CAGACCATGG	CACTCCTGCC	1200
	TCAGCAGATT	TTTTTCATAA	ATATGTCCGT	TATGTGAGGA	AGAGCCACAT	TACAGGACCC	1260
	CTCCTTGTTT	CCATCGATGC	TGGTGTAGGC	CGAACAGGGG	TGTTTCATATG	TGTGGATGTT	1320
60	GTGTTCTCTG	ACTGCAGAGAA	GAACTACTCT	TTTGACATTA	TGAACATAGT	GACCCAGATG	1380
	AGAAAGCAGC	GCTGTGGCAT	GATTCAAACC	AAGGTTACCG	AGCAGCTTGG	AGCCTGAGCC	1440
	GTGCTGAAGC	GTCTGCGGGC	CGTGCAGTCT	GCCTTCTGAT	TTTTCTCTCT	GAAAGTCCCT	1500
	GAAGGTAGCA	CTACTGGGCA	CAGAGTGAAC	TGTTTCCACT	TGATCTTTCT	GAACAAGAGC	1560
	AAAATACCCCT	CCATGCGCTT	TACGGAACAG	GAAGTTGCAT	GAAACAACCT	CCGCTTGCTT	1620
65	GTCTGGTTTG	TGGTATTACA	GAGCTTAATA	AAAGACTTAG	ATGTGAAAAA	AAAAAAAAAA	1680

AAAAAAAAAA AA

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(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 320 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GAAAATAATT GTAATGTTAT TGCTATGATA ACCAGAGAGA TAGAAGGTGG AGTTATCAAG 60
TGTTGCAGTT ACTGGCCCGT TTCTCTGAAG GAGCCTTTGG AATTCAAACA CTTTCATGTC 120
CTTCTGGAGA ACTTTCAGAT AACTCAGTAT TTTGTCATCC GAATATTTCA AATTGTGAAG 180
AAGTCCACAG GAAAGAGTCA CTCTGTAAAA CACTTGCAGT TCATCAAATG GCCAGACCAT 240
GGCACTCCTG CCTCAGTAGA TTTTTCATC AAATATGTCC GTTATGTGAG GAAGAGCCAC 300
ATTACAGGAC CCCTCCTTGT 320

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 4456 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GGCACGAGAG GAGCAGCAGA AGTTCGGGGA GCGGGTTGCA TACTTCCAGA GCGCCCTGGA 60
CAAGCTCAAT GAAGCCATCA AGTTGGCCAA GGGCCAGCCT GACACTGTGC AAGACGCGCT 120
TCGCTTCACT ATGGATGTCA TTGGGGGAAA GTACAATTCT GCCAAGAAGG ACAACGACTT 180
CATTTACCAT GAGGCTGTCC CAGCATTGAC ACCCTTCAGC CTGTAAAAGG AGCCCCCTTG 240
GTGAAGCCCT TGCCAGTGAA CCCACAGAC CCAGCTGTTA CAGGCCCTGA CATCTTTGCC 300
AAACTGGTAC CCATGGCTGC CCACGAGGCC TCGTCACTGT ACAGTGAGGA GAAGGCCAAG 360
CTGCTCCGGG AGATGATGGC CAAGATTGAG GACAAGAATG AGGTCCTGGA CCAGTTCATG 420
GATTCAATGC AGTTGGATCC CGAGACGGTG GACAACCTTG ATGCCTACAG CCACATCCCA 480
CCCCAGCTCA TGGAGAAGTG CGCGGCTCTC AGCGTCCGGC CCGACACTGT CAGGAACCTT 540
GTACAGTCCA TGCAAGTGCT GTCAGGTGTG TTCACGGATG TGGAGGCTTC CCTGAAGGAC 600
ATCAGAGATC TGTGGAGGA GGATGAGCTG CTAGAGCAGA AGTTTCAGGA GGCGGTGGGC 660
CAGGCAGGGG CCATCTCCAT CACCTCCAAG GCTGAGCTGG CAGAGGTGAG GCGAGAATGG 720
GCCAAGTACA TGAAGTCCA TGAGAAGGCC TCCTTACCA ACAGTGAGCT GCACCGTGCC 780
ATGAACCTGC ACGTCGGCAA CCTGCGCCTG CTCAGCGGGC CGCTTGACCA GGTCCGGGCT 840
GCCCTGCCCA CACCGGCCCT CTCCCCAGAG GACAAGGCCG TGCTGCAAAA CCTAAAGCGC 900
ATCCTGGCTA AGGTGCAGGA GATGCGGGAC CAGCGCGTGT CCCTGGAGCA GCAGCTGCGT 960
GAGCTTATCC AGAAAGATGA CATCACTGCC TCGCTGGTCA CCACAGACCA CTCAGAGATG 1020
AAGAAGTTGT TCGAGGAGCA GCTGAAAAAG TATGACCAGC TGAAGGTGTA CCTGGAGCAG 1080
AACCTGGCCG CCCAGGACCG TGCTCTCTGT GCACTGACAG AGGCCAACGT GCAGTACGCA 1140
GCCGTGCGGC GGGTACTCAG CGACTTGGAC CAAAAGTGGA ACTCCACGCT GCAGACCTTG 1200
GTGGCCTCGT ATGAAGCCTA TGAGGACCTG ATGAAGAAGT CGCAGGAGGG CAGGGACTTC 1260
TACGCAGATC TGGAGAGCAA GGTGGCTGCT CTGCTGGAGC GCACGCAGTC CACCTGCCAG 1320
GCCCAGCAGG CTGCCCAGCA GCAGCTCCTG GACAGGGAGC TGAAGAAGAA GCCGCCGCCA 1380
CGGCCACAG CCCCAAAGCC GCTGCTGCCC CGCAGGGAGG AGAGTGAGGC AGTGGAAGCA 1440
GGAGACCCCT CTGAGGAGCT GCGCAGCCTC CCCCCTGACA TGGTGGCTGG CCCACGACTG 1500
CCTGACACCT TCGTGGGAAG TGCCACCCCG CTCCACTTTC CTCCAGCCC CTTCCCCAGC 1560
TCCACAGGCC CAGGACCCCA CTATCTCTCA GGCCCCCTTG CCCCTGGTAC CTACTCGGGC 1620
CCCACCCAGC TGATACAGCC CAGGGCCCCA GGGCCCCATG CAATGCCCGT AGCACCTGGG 1680
CCTGCCCTCT ACCCAGCCCC TGCTTACACA CCGGAGCTGG GCCTTGTGCC CCGATCCTCC 1740
CCACAGCATG CGGTGGTAG CAGTCCCTAT TTGGGGGTAG GGCCGGCCCC ACCAGTTGCA 1800
GGTCTCCCTT GCGCCCCACC TCCTCAATTG TCAGGCCCG AGTTGGCCAT GGCGGTTCCG 1860
CCAGCCACCA CCACAGTAGA TAGCATCCAG GCGCCCATCC CCAGCCACAC AGCCCCACGG 1920

150

CCAAACCCCA CCCCTGCTCC TCCCCCGCCC TGCTTCCCTG TGCCCCCACC GCAGCCACTG 1980
 CCCACGCCTT ACACCTACCC TGCAGGGGCT AAGCAACCCA TCCCAGCACA GCACCACTTC 2040
 TCTTCTGGGA TCCCCACAGG TTTTCCAGCC CCAAGGATTG GGCCCCAGCC CCAGCCCCAT 2100
 5 CCTCAGCCCC ATCCTTCACA AGCGTTTGGG CCTCAGCCCC CACAGCAGCC CCTTCCACTC 2160
 CAGCATCCAC ATCTCTTCCC ACCCCAGGCC CCAGGACTCC TACCCCAACA ATCCCCCTAC 2220
 CCCTATGCCC CTCAGCCTGG GGTCCTGGGG CAGCCGCCAC CCCCCCTACA CACCCAGCTC 2280
 TACCCAGGTC CCGCTCAAGA CCCTCTGCCA GCCCCTCAG GGGCTCTGCC TTTCCCCAGC 2340
 CCTGGGCCCC CTCAGCCTCC CCATCCCCCA CTGGCATATG GTCCTGCCCC TTCTACCAGA 2400
 CCCATGGGCC CCCAGGCAGC CCCTCTTACC ATTCGAGGGC CCTCGTCTGC TGGCCAGTCC 2460
 10 ACCCCTAGTC CCCACCTGGT GCCTTCACCT GCCCATCTC CAGGGCCTGG TCCGGTACCC 2520
 CCTCGCCCCC CAGCAGCAGA ACCACCCCTT TGCCTGCGCC GAGGCGCCGC AGCTGCAGAC 2580
 CTGCTCTCCT CCAGCCCGGA GAGCCAGCAT GGCGGCACTC AGTCTCCTGG GGGTGGGCAG 2640
 CCCCTGCTGC AGCCCACCAA GGTGGATGCA GCTGAGGGTC GTCGGCCGCA GGCCCTGCGG 2700
 CTGATTGAGC GGGACCCCTA TGAGCATCCT GAGAGGCTGC GGCAGTTGCA GCAGGAGCTG 2760
 15 GAGGCCTTTT GGGGTACAGT GGGGGATGTG GGAGCTCTGG ACACTGTCTG GCGAGAGCTG 2820
 CAAGATGCGC AGGAACATGA TGCCCAGGCG CGTTCCATCG CCATTGCCCC CTGCTACTCA 2880
 CTGAAGAACC GGCACCAGGA TGTCATGCCC TATGACAGTA ACCGTGTGGT GCTGCGCTCA 2940
 GGCAAGGATG ACTACATCAA TGCCAGCTGC GTGGAGGGGC TCTCCCCATA CTGCCCCCGG 3000
 CTAGTGGCAA CCCAGGCCCC ACTGCCTGGC ACAGCTGCTG ACTTCTGGCT CATGGTCCAT 3060
 20 GAGCAGAAAG TGTCACTCAT TGTCATGCTG GTTCTGAGG CTGAGATGGA GAAGCAAAAA 3120
 GTGGCAGCT ACTTCCCCAC CGAGAGGGGC CAGCCCATGG TGCACGGTGC CCTGAGCCTG 3180
 GCATTGAGCA GCGTCCGAG CACCGAAACC CATGTGGAGC GCGTGCTGAG CCTGCAGTTC 3240
 CGAGACCAGA GCCTCAAGCG CTCTCTTGTG CACCTGCACT TCCCCACTTG GCCTGAGTTA 3300
 GGCTGCCCCG ACAGCCCCAG CAACTTGCTG CGCTTCATCC AGGAGGTGCA CGCACATTAC 3360
 25 CTGCATCAGC GGCCGCTGCA CACGCCCATC ATTGTGCACT GCAGCTCTGG TGTGGGCCGC 3420
 ACGGGAGCCT TTGCACTGCT CTATGCAGCT GTGCAGGAGG TGGAGGCTGG GAACGGAATC 3480
 CCTGAGCTGC CTCAGCTGGT GCGGCGCATG CGGCAGCAGA GAAAGCACAT GCTGCAGGAG 3540
 AAGCTGCACC TCAGGTTCTG CTATGAGGCA GTGGTGAGAC ACGTGAGCA GGTCTGCAG 3600
 CGCCATGGTG TGCCCTCTCC ATGCAAACCC TTGGCCAGTG CAAGCATCAG CCAGAAGAAC 3660
 30 CACCTTCCCTC AGGACTCCCA GGACCTGGTC CTCGGTGGGG ATGTGCCAT CAGCTCCATC 3720
 CAGGCCACCA TTGCCAAGCT CAGCATTCGG CCTCCTGGGG GGTGGAGTC CCCGGTTGCC 3780
 AGCTTGCCAG GCCCTGCAGA GCCCCCAGGC CTCCCGCCAG CCAGCCTCCC AGAGTCTACC 3840
 CCAATCCCAT CTTCCTCCCC ACCCCCCCTT TCCTCCCCAC TACCTGAGGC TCCCCAGCCT 3900
 AAGGAGGAGC CGCCAGTGCC TGAAGCCCC AGCTCGGGGC CCCCCTCCTC CTCCTGGAA 3960
 35 TTGCTGGCCT CTTTGACCCC AGAGGCCCTT TCCTGGACA GCTCCCTGCG GGGCAACAG 4020
 CGGATGAGCA AGCATAACTT TCTGCAGGCC CATAACGGGC AAGGGCTGCG GGCCACCCGG 4080
 CCTCTGACG ACCCCCTCAG CCTTCTGGAT CCACTCTGGA CACTCAACAA GACCTGAACA 4140
 GGTTTTGCTT ACCTGGTCCT TACACTACAT CATCATCATC TCATGCCCAC CTGCCCACAC 4200
 CCAGCAGGCT TTCTCAGTGG GCACAGTCTC TTACTCCCAT TTCTGCTGCC TTTGGCCCTG 4260
 40 CCTGGCCCAG CCTGCACCCC TGTGGGGTGG AAATGTACTG CAGGCTCTGG GTCAGTTCT 4320
 GCTCCTTTAT GGGACCCGAC ATTTTTCAGC TCTTTGCTAT TGAAATAATA AACCACCCTG 4380
 TTCTGTGAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA 4440
 AAAAAAAAAA AAAAAA 4456

45

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

50

(A) LENGTH: 1793 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

60

65

CGGCCACACT GACTAGAGCC AACC CGGCAC TTCAAAGGG TGTCGGTGCC GCGCTCCCTT 60
 CCCGCGCCCC GGGAACCTCA AAGCGGGCCG TGCTGCCCGG GCTGCCTCGC TCTGCTCTGG 120
 GGCTCTCGAG CCCGCGCGCG GCGCCTGGT GGCGATGACC CGGGCGCTCT GCTCAGCGCT 180
 CCGCAGGCT CTCCTGCTGC TCGCAGCGGC CGCCGAGCTC TCGCCAGGAC TGAAGTGTGT 240
 ATGTCTTTTG TGTGATTCTT CAAACTTTAC CTGCCAAACA GAAGGAGCAT GTTGGGCATC 300
 AGTCATGCTA ACCAATGGAA AAGAGCAGT GATCAAATCC TGTGTCTCCC TTCCAGAACT 360
 GAATGCTCAA GTCTTCTGTC ATAGTTCCAA CAATGTTACC AAAACCGAAT GCTGCTTCAC 420
 AGATTTTTCG AACAACATAA CACTGCACCT TCCAACAGCA TCACCAAATG CCCCCAACT 480

151

TGGACCCATG GAGCTGGCCA TCATTATTAC TGTGCCTGTT TGCCTCCTGT CCATAGCTGC 540
 GATGCTGACA GTATGGGCAT GCCAGGGTCG ACAGTGCTCC TACAGGAAGA AAAAGAGACC 600
 AAATGTGGAG GAACCACTCT CTGAGTGCAA TCTGGTAAAT GCTGGAAAAA CTCTGAAAGA 660
 TCTGATTTAT GATGTGACCG CCTCTGGATC TGGCTCTGGT CTACCTCTGT TGGTTCAAAG 720
 5 GACAATTGCA AGGACGATTG TGCTTCAGGA AATAGTAGGA AAAGGTAGAT TTGGTGAGGT 780
 GTGGCATGGA AGATGGTGTG GGGAAAGATGT GGCTGTGAAA ATATTCTCCT CCAGAGATGA 840
 AAGATCTTGG TTTTCGTGAGG CAGAAATTTA CCAGACGGTC ATGCTGCGAC ATGAAAACAT 900
 CCTTGGTTTTT ATTGCTGCTG ACAACAAAGA TAATGGAAC TGGACTCAAC TTTGGCTGGT 960
 ATCTGAATAT CATGAACAGG GCTCCTTATA TGACTATTTG AATAGAAATA TAGTGACCGT 1020
 10 GGCTGGAATG ATCAAGCTGG CGCTCTCAAT TGCTAGTGGT CTGGCACACC TTCATATGGA 1080
 GATTGTTGGT ACACAAGGTA AACCTGCTAT TGCTCATCGA GACATAAAAT CAAAGAATAT 1140
 CTTAGTGAAA AAGTGTGAAA CTTGTGCCAT AGCGGACTTA GGGTTGGCTG TGAAGCATGA 1200
 TTCAATACTG AACACTATCG ACATACCTCA GAATCCTAAA GTGGGAACCA AGAGGTATAT 1260
 GGCTCCTGAA ATGCTTGATG ATACAATGAA TGTGAATATC TTTGAGTCCT TCAAACGAGC 1320
 15 TGACATCTAT TCTGTTGGTC TGGTTTACTG GGAAATAGCC CGGAGGTGTT CAGTCGGAGG 1380
 AATTGTTGAG GAGTACCAAT TGCCTTATTA TGACATGGTG CCTTCAGATC CCTCGATAGA 1440
 GGAAATGAGA AAGGTTGTTT GTGACCAGAA GTTTCGACCA AGTATCCCAA ACCAGTGGCA 1500
 AAGTTGTGAA GCACTCCGAG TCATGGGGAG AATAATGCGT GAGTGTTGGT ATGCCAACGG 1560
 AGCGGCCCGC CTAACGCTC TTCGTATTAA GAAGACTATA TCTCAACTTT GTGTCAAAGA 1620
 20 AGACTGCAAA GCCTAATGAT GATAATTATG TTAAAAAGAA ATCTCTCATA GCTTCTTTT 1680
 CCATTTTCCC CTTTATGTGA ATGTTTTTGC CATTTTTTTT TTGTTCTACC TCAAAGATAA 1740
 GACAGTACAG TATTTAAGTG CCCATAAGGC AGCATGAAA GATAACTCTA AAG 1793

25

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 807 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

40

Met Asp Gln Arg Glu Ile Leu Gln Lys Phe Leu Asp Glu Ala Gln Ser
 1 5 10 15

Lys Lys Ile Thr Lys Glu Glu Phe Ala Asn Glu Phe Leu Lys Leu Lys
 20 25 30

45

Arg Gln Ser Thr Lys Tyr Lys Ala Asp Lys Thr Tyr Pro Thr Thr Val
 35 40 45

50

Ala Glu Lys Pro Lys Asn Ile Lys Lys Asn Arg Tyr Lys Asp Ile Leu
 50 55 60

Pro Tyr Asp Tyr Ser Arg Val Glu Leu Ser Leu Ile Thr Ser Asp Glu
 65 70 75 80

55

Asp Ser Ser Tyr Ile Asn Ala Asn Phe Ile Lys Gly Val Tyr Gly Pro
 85 90 95

Lys Ala Tyr Ile Ala Thr Gln Gly Pro Leu Ser Thr Thr Leu Leu Asp
 100 105 110

60

Phe Trp Arg Met Ile Trp Glu Tyr Ser Val Leu Ile Ile Val Met Ala
 115 120 125

Cys Met Glu Tyr Glu Met Gly Lys Lys Lys Cys Glu Arg Tyr Trp Ala
 130 135 140

65

152

	Glu	Pro	Gly	Glu	Met	Gln	Leu	Glu	Phe	Gly	Pro	Phe	Ser	Val	Ser	Cys	145	150	155	160
5	Glu	Ala	Glu	Lys	Arg	Lys	Ser	Asp	Tyr	Ile	Ile	Arg	Thr	Leu	Lys	Val	165	170		175
	Lys	Phe	Asn	Ser	Glu	Thr	Arg	Thr	Ile	Tyr	Gln	Phe	His	Tyr	Lys	Asn	180	185		190
10	Trp	Pro	Asp	His	Asp	Val	Pro	Ser	Ser	Ile	Asp	Pro	Ile	Leu	Glu	Leu	195	200	205	
	Ile	Trp	Asp	Val	Arg	Cys	Tyr	Gln	Glu	Asp	Asp	Ser	Val	Pro	Ile	Cys	210	215	220	
15	Ile	His	Cys	Ser	Ala	Gly	Cys	Gly	Arg	Thr	Gly	Val	Ile	Cys	Ala	Ile	225	230	235	240
20	Asp	Tyr	Thr	Trp	Met	Leu	Leu	Lys	Asp	Gly	Ile	Ile	Pro	Glu	Asn	Phe	245	250		255
	Ser	Val	Phe	Ser	Leu	Ile	Arg	Glu	Met	Arg	Thr	Gln	Arg	Pro	Ser	Leu	260	265		270
25	Val	Gln	Thr	Gln	Glu	Gln	Tyr	Glu	Leu	Val	Tyr	Asn	Ala	Val	Leu	Glu	275	280		285
	Leu	Phe	Lys	Arg	Gln	Met	Asp	Val	Ile	Arg	Asp	Lys	His	Ser	Gly	Thr	290	295	300	
30	Glu	Ser	Gln	Ala	Lys	His	Cys	Ile	Pro	Glu	Lys	Asn	His	Thr	Leu	Gln	305	310	315	320
35	Ala	Asp	Ser	Tyr	Ser	Pro	Asn	Leu	Pro	Lys	Ser	Thr	Thr	Lys	Ala	Ala	325	330		335
	Lys	Met	Met	Asn	Gln	Gln	Arg	Thr	Lys	Met	Glu	Ile	Lys	Glu	Ser	Ser	340	345		350
40	Ser	Phe	Asp	Phe	Arg	Thr	Ser	Glu	Ile	Ser	Ala	Lys	Glu	Glu	Leu	Val	355	360		365
	Leu	His	Pro	Ala	Lys	Ser	Ser	Thr	Ser	Phe	Asp	Phe	Leu	Glu	Leu	Asn	370	375	380	
45	Tyr	Ser	Phe	Asp	Lys	Asn	Ala	Asp	Thr	Thr	Met	Lys	Trp	Gln	Thr	Lys	385	390	395	400
50	Ala	Phe	Pro	Ile	Val	Gly	Glu	Pro	Leu	Gln	Lys	His	Gln	Ser	Leu	Asp	405	410		415
	Leu	Gly	Ser	Leu	Leu	Phe	Glu	Gly	Cys	Ser	Asn	Ser	Lys	Pro	Val	Asn	420	425		430
55	Ala	Ala	Gly	Arg	Tyr	Phe	Asn	Ser	Lys	Val	Pro	Ile	Thr	Arg	Thr	Lys	435	440		445
	Ser	Thr	Pro	Phe	Glu	Leu	Ile	Gln	Gln	Arg	Glu	Thr	Lys	Glu	Val	Asp	450	455	460	
60	Ser	Lys	Glu	Asn	Phe	Ser	Tyr	Leu	Glu	Ser	Gln	Pro	His	Asp	Ser	Cys	465	470	475	480
65	Phe	Val	Glu	Met	Gln	Ala	Gln	Lys	Val	Met	His	Val	Ser	Ser	Ala	Glu	485	490		495

153

Leu Asn Tyr Ser Leu Pro Tyr Asp Ser Lys His Gln Ile Arg Asn Ala
 500 505 510
 5 Ser Asn Val Lys His His Asp Ser Ser Ala Leu Gly Val Tyr Ser Tyr
 515 520 525
 Ile Pro Leu Val Glu Asn Pro Tyr Phe Ser Ser Trp Pro Pro Ser Gly
 530 535 540
 10 Thr Ser Ser Lys Met Ser Leu Asp Leu Pro Glu Lys Gln Asp Gly Thr
 545 550 555 560
 Val Phe Pro Ser Ser Leu Leu Pro Thr Ser Ser Thr Ser Leu Phe Ser
 565 570 575
 Tyr Tyr Asn Ser His Asp Ser Leu Ser Leu Asn Ser Pro Thr Asn Ile
 580 585 590
 20 Ser Ser Leu Leu Asn Gln Glu Ser Ala Val Leu Ala Thr Ala Pro Arg
 595 600 605
 Ile Asp Asp Glu Ile Pro Pro Pro Leu Pro Val Arg Thr Pro Glu Ser
 610 615 620
 25 Phe Ile Val Val Glu Glu Ala Gly Glu Phe Ser Pro Asn Val Pro Lys
 625 630 635 640
 Ser Leu Ser Ser Ala Val Lys Val Lys Ile Gly Thr Ser Leu Glu Trp
 645 650 655
 Gly Gly Thr Ser Glu Pro Lys Lys Phe Asp Asp Ser Val Ile Leu Arg
 660 665 670
 35 Pro Ser Lys Ser Val Lys Leu Arg Ser Pro Lys Ser Glu Leu His Gln
 675 680 685
 Asp Arg Ser Ser Pro Pro Pro Pro Leu Pro Glu Arg Thr Leu Glu Ser
 690 695 700
 40 Phe Phe Leu Ala Asp Glu Asp Cys Met Gln Ala Gln Ser Ile Glu Thr
 705 710 715 720
 Tyr Ser Thr Ser Tyr Pro Asp Thr Met Glu Asn Ser Thr Ser Ser Lys
 725 730 735
 45 Gln Thr Leu Lys Thr Pro Gly Lys Ser Phe Thr Arg Ser Lys Ser Leu
 740 745 750
 50 Lys Ile Leu Arg Asn Met Lys Lys Ser Ile Cys Asn Ser Cys Pro Pro
 755 760 765
 Asn Lys Pro Ala Glu Ser Val Gln Ser Asn Asn Ser Ser Ser Phe Leu
 770 775 780
 55 Asn Phe Gly Phe Ala Asn Arg Phe Ser Lys Pro Lys Gly Pro Arg Asn
 785 790 795 800
 60 Pro Pro Pro Thr Trp Asn Ile
 805
 65

154

(2) INFORMATION FOR SEQ ID NO: 10:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 488 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

15 Met Glu Pro Phe Leu Arg Arg Arg Leu Ala Phe Leu Ser Phe Phe Trp
 1 5 10 15
 Asp Lys Ile Trp Pro Ala Gly Gly Glu Pro Asp His Gly Thr Pro Gly
 20 25 30
 20 Ser Leu Asp Pro Asn Thr Asp Pro Val Pro Thr Leu Pro Ala Glu Pro
 35 40 45
 25 Cys Ser Pro Phe Pro Gln Leu Phe Leu Ala Leu Tyr Asp Phe Thr Ala
 50 55 60
 Arg Cys Gly Gly Glu Leu Ser Val Arg Arg Gly Asp Arg Leu Cys Ala
 65 70 75 80
 30 Leu Glu Glu Gly Gly Gly Tyr Ile Phe Ala Arg Arg Leu Ser Gly Gln
 85 90 95
 Pro Ser Ala Gly Leu Val Pro Ile Thr His Val Ala Lys Ala Ser Pro
 100 105 110
 35 Glu Thr Leu Ser Asp Gln Pro Trp Tyr Phe Ser Gly Val Ser Arg Thr
 115 120 125
 40 Gln Ala Gln Gln Leu Leu Leu Ser Pro Pro Asn Glu Pro Gly Ala Phe
 130 135 140
 Leu Ile Arg Pro Ser Glu Ser Ser Leu Gly Gly Tyr Ser Leu Ser Val
 145 150 155 160
 45 Arg Ala Gln Ala Lys Val Cys His Tyr Arg Val Ser Met Ala Ala Asp
 165 170 175
 Gly Ser Leu Tyr Leu Gln Lys Gly Arg Leu Phe Pro Gly Leu Glu Glu
 180 185 190
 50 Leu Leu Thr Tyr Tyr Lys Ala Asn Trp Lys Leu Ile Gln Asn Pro Leu
 195 200 205
 55 Leu Gln Pro Cys Met Pro Gln Lys Ala Pro Arg Gln Asp Val Trp Glu
 210 215 220
 Arg Pro His Ser Glu Phe Ala Leu Gly Arg Lys Leu Gly Glu Gly Tyr
 225 230 235 240
 60 Phe Gly Glu Val Trp Glu Gly Leu Trp Leu Gly Ser Leu Pro Val Ala
 245 250 255
 Ile Lys Val Ile Lys Ser Ala Asn Met Lys Leu Thr Asp Leu Ala Lys
 260 265 270
 65

155

Glu Ile Gln Thr Leu Lys Gly Leu Arg His Glu Arg Leu Ile Arg Leu
 275 280 285
 5 His Ala Val Cys Ser Gly Gly Glu Pro Val Tyr Ile Val Thr Glu Leu
 290 295 300
 Met Arg Lys Gly Asn Leu Gln Ala Phe Leu Gly Thr Pro Glu Gly Arg
 305 310 315 320
 10 Ala Leu Arg Leu Pro Pro Leu Leu Gly Phe Ala Cys Gln Val Ala Glu
 325 330 335
 Gly Met Ser Tyr Leu Glu Glu Gln Arg Val Val His Arg Asp Leu Ala
 340 345 350
 15 Ala Arg Asn Val Leu Val Asp Asp Gly Leu Ala Cys Lys Val Ala Asp
 355 360 365
 20 Phe Gly Leu Ala Arg Leu Leu Lys Asp Asp Ile Tyr Ser Pro Ser Ser
 370 375 380
 Ser Ser Lys Ile Pro Val Lys Trp Thr Ala Pro Glu Ala Ala Asn Tyr
 385 390 395 400
 25 Arg Val Phe Ser Gln Lys Ser Asp Val Trp Ser Phe Gly Val Leu Leu
 405 410 415
 30 His Glu Val Phe Thr Tyr Gly Gln Cys Pro Tyr Glu Gly Met Thr Asn
 420 425 430
 His Glu Thr Leu Gln Gln Ile Met Arg Gly Tyr Arg Leu Pro Arg Pro
 435 440 445
 35 Ala Ala Cys Pro Ala Glu Val Tyr Val Leu Met Leu Glu Cys Trp Arg
 450 455 460
 Ser Ser Pro Glu Glu Arg Pro Ser Phe Ala Thr Leu Arg Glu Lys Leu
 465 470 475 480
 40 His Ala Ile His Arg Cys His Pro
 485

45 (2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 426 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Ser Ser Pro Arg Lys Val Arg Gly Lys Thr Gly Arg Asp Asn Asp
 1 5 10 15
 60 Glu Glu Glu Gly Asn Ser Gly Asn Leu Asn Leu Arg Asn Ser Leu Pro
 20 25 30
 65 Ser Ser Ser Gln Lys Met Thr Pro Thr Lys Pro Ile Phe Gly Asn Lys
 35 40 45

156

	Met	Asn	Ser	Glu	Asn	Val	Lys	Pro	Ser	His	His	Leu	Ser	Phe	Ser	Asp	
	50						55					60					
5	Lys	Tyr	Glu	Leu	Val	Tyr	Pro	Glu	Pro	Leu	Glu	Ser	Asp	Thr	Asp	Glu	
	65					70					75					80	
	Thr	Val	Trp	Asp	Val	Ser	Asp	Arg	Ser	Leu	Arg	Asn	Arg	Trp	Asn	Ser	
					85					90					95		
10	Met	Asp	Ser	Glu	Thr	Ala	Gly	Pro	Ser	Lys	Thr	Val	Ser	Pro	Val	Leu	
				100				105						110			
	Ser	Gly	Ser	Ser	Arg	Leu	Ser	Lys	Asp	Thr	Glu	Thr	Ser	Val	Ser	Glu	
15			115					120					125				
	Lys	Glu	Leu	Thr	Gln	Leu	Ala	Gln	Ile	Arg	Pro	Leu	Ile	Phe	Asn	Ser	
	130						135					140					
20	Ser	Ala	Arg	Ser	Ala	Met	Arg	Asp	Cys	Leu	Asn	Thr	Leu	Gln	Lys	Lys	
	145					150					155					160	
	Glu	Glu	Leu	Asp	Ile	Ile	Arg	Glu	Phe	Leu	Glu	Leu	Glu	Gln	Met	Thr	
25					165					170					175		
	Leu	Pro	Asp	Asp	Phe	Asn	Ser	Gly	Asn	Thr	Leu	Gln	Asn	Arg	Asp	Lys	
				180					185					190			
30	Asn	Arg	Tyr	Arg	Asp	Ile	Leu	Pro	Tyr	Asp	Ser	Thr	Arg	Val	Pro	Leu	
			195					200					205				
	Gly	Lys	Asn	Lys	Asp	Tyr	Ile	Asn	Ala	Ser	Tyr	Ile	Arg	Ile	Val	Asn	
	210						215					220					
35	His	Glu	Glu	Glu	Tyr	Phe	Tyr	Ile	Ala	Thr	Gln	Gly	Pro	Leu	Pro	Glu	
	225					230					235					240	
	Thr	Ile	Glu	Asp	Phe	Trp	Gln	Met	Val	Leu	Glu	Asn	Asn	Cys	Asn	Val	
40					245					250					255		
	Ile	Ala	Met	Ile	Thr	Arg	Glu	Ile	Glu	Cys	Gly	Val	Ile	Lys	Cys	Tyr	
				260					265					270			
45	Ser	Tyr	Trp	Pro	Ile	Ser	Leu	Lys	Glu	Pro	Leu	Glu	Phe	Glu	His	Phe	
			275					280					285				
	Ser	Val	Phe	Leu	Glu	Thr	Phe	His	Val	Thr	Gln	Tyr	Phe	Thr	Val	Arg	
			290				295					300					
50	Val	Phe	Gln	Ile	Val	Lys	Lys	Ser	Thr	Gly	Lys	Ser	Gln	Cys	Val	Lys	
	305					310					315					320	
	His	Leu	Gln	Phe	Thr	Lys	Trp	Pro	Asp	His	Gly	Thr	Pro	Ala	Ser	Ala	
55					325					330					335		
	Asp	Phe	Phe	Ile	Lys	Tyr	Val	Arg	Tyr	Val	Arg	Lys	Ser	His	Ile	Thr	
				340					345					350			
60	Gly	Pro	Leu	Leu	Val	His	Cys	Ser	Ala	Gly	Val	Gly	Arg	Thr	Gly	Val	
			355					360					365				
	Phe	Ile	Cys	Val	Asp	Val	Val	Phe	Ser	Ala	Ile	Glu	Lys	Asn	Tyr	Ser	
							375					380					
65																	

157

Phe Asp Ile Met Asn Ile Val Thr Gln Met Arg Lys Gln Arg Cys Gly
385 390 395 400

Met Ile Gln Thr Lys Glu Gln Tyr Gln Phe Cys Tyr Glu Ile Val Leu
405 410 415

Glu Val Leu Gln Asn Leu Leu Ala Leu Tyr
420 425

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 463 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Met Ser Ser Pro Arg Lys Val Arg Gly Lys Thr Gly Arg Asp Asn Asp
1 5 10 15

Glu Glu Glu Gly Asn Ser Gly Asn Leu Asn Leu Arg Asn Ser Leu Pro
20 25 30

Ser Ser Ser Gln Lys Met Thr Pro Thr Lys Pro Val Gln Asn Lys Asn
35 40 45

Leu Met Lys Tyr Glu Glu His Leu Asp Ile Leu Met Val Phe Leu Leu
50 55 60

Ile Lys Thr Ile Trp Tyr Asn Val Phe Lys Leu Trp Lys Gly Lys Leu
65 70 75 80

Ile Phe Gly Asn Lys Met Asn Ser Glu Asn Val Lys Pro Ser His His
85 90 95

Leu Ser Phe Ser Asp Lys Tyr Glu Leu Val Tyr Pro Glu Pro Leu Glu
100 105 110

Ser Asp Thr Asp Glu Thr Val Trp Asp Val Ser Asp Arg Ser Leu Arg
115 120 125

Asn Arg Trp Asn Ser Met Asp Ser Glu Thr Ala Gly Pro Ser Lys Thr
130 135 140

Val Ser Pro Val Leu Ser Gly Ser Ser Arg Leu Ser Lys Asp Thr Glu
145 150 155 160

Thr Ser Val Ser Glu Lys Glu Leu Thr Gln Leu Ala Gln Ile Arg Pro
165 170 175

Leu Ile Phe Asn Ser Ser Ala Arg Ser Ala Met Arg Asp Cys Leu Asn
180 185 190

Thr Leu Gln Lys Lys Glu Glu Leu Asp Ile Ile Arg Glu Phe Leu Glu
195 200 205

Leu Glu Gln Met Thr Leu Pro Asp Asp Phe Asn Ser Gly Asn Thr Leu
210 215 220

158

Gln Asn Arg Asp Lys Asn Arg Tyr Arg Asp Ile Leu Pro Tyr Asp Ser
 225 230 235 240
 5 Thr Arg Val Pro Leu Gly Lys Asn Lys Asp Tyr Ile Asn Ala Ser Tyr
 245 250 255
 Ile Arg Ile Val Asn His Glu Glu Glu Tyr Phe Tyr Ile Ala Thr Gln
 260 265 270
 10 Gly Pro Leu Pro Glu Thr Ile Glu Asp Phe Trp Gln Met Val Leu Glu
 275 280 285
 Asn Asn Cys Asn Val Ile Ala Met Ile Thr Arg Glu Ile Glu Cys Gly
 290 295 300
 Val Ile Lys Cys Tyr Ser Tyr Trp Pro Ile Ser Leu Lys Glu Pro Leu
 305 310 315 320
 20 Glu Phe Glu His Phe Ser Val Phe Leu Glu Thr Phe His Val Thr Gln
 325 330 335
 Tyr Phe Thr Val Arg Val Phe Gln Ile Val Lys Lys Ser Thr Gly Lys
 340 345 350
 25 Ser Gln Cys Val Lys His Leu Gln Phe Thr Lys Trp Pro Asp His Gly
 355 360 365
 Thr Pro Ala Ser Ala Asp Phe Phe Ile Lys Tyr Val Arg Tyr Val Arg
 370 375 380
 Lys Ser His Ile Thr Gly Pro Leu Leu Val His Cys Ser Ala Gly Val
 385 390 395 400
 35 Gly Arg Thr Gly Val Phe Ile Cys Val Asp Val Val Phe Ser Ala Ile
 405 410 415
 Glu Lys Asn Tyr Ser Phe Asp Ile Met Asn Ile Val Thr Gln Met Arg
 420 425 430
 40 Lys Gln Arg Cys Gly Met Ile Gln Thr Lys Glu Gln Tyr Gln Phe Cys
 435 440 445
 Tyr Glu Ile Val Leu Glu Val Leu Gln Asn Leu Leu Ala Leu Tyr
 450 455 460

(2) INFORMATION FOR SEQ ID NO: 13:

50 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 405 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 55 (D) TOPOLOGY: linear
 (ii) MOLECULE TYPE: peptide
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
 60 Met Ser Ser Pro Arg Lys Val Arg Gly Lys Thr Gly Arg Asp Asn Asp
 1 5 10 15
 65 Glu Glu Glu Gly Asn Ser Gly Asn Leu Asn Leu Arg Asn Ser Leu Pro
 20 25 30

5 Ser Ser Ser Gln Lys Met Thr Pro Thr Lys Pro Ile Phe Gly Asn Lys
 35 40 45
 Met Asn Ser Glu Asn Val Lys Pro Ser His His Leu Ser Phe Ser Asp
 50 55 60
 10 Lys Tyr Glu Leu Val Tyr Pro Glu Pro Leu Glu Ser Asp Thr Asp Glu
 65 70 75 80
 Thr Val Trp Asp Val Ser Asp Arg Ser Leu Arg Asn Arg Trp Asn Ser
 85 90 95
 15 Met Asp Ser Glu Thr Ala Gly Pro Ser Lys Thr Val Ser Pro Val Leu
 100 105 110
 Ser Gly Ser Ser Arg Leu Ser Lys Asp Thr Glu Thr Ser Val Ser Glu
 115 120 125
 20 Lys Glu Leu Thr Gln Leu Ala Gln Ile Arg Pro Leu Ile Phe Asn Ser
 130 135 140
 Ser Ala Arg Ser Ala Met Arg Asp Cys Leu Asn Thr Leu Gln Lys Lys
 145 150 155 160
 Glu Glu Leu Asp Ile Ile Arg Glu Phe Leu Glu Leu Glu Gln Met Thr
 165 170 175
 30 Leu Pro Asp Asp Phe Asn Ser Gly Asn Thr Leu Gln Asn Arg Asp Lys
 180 185 190
 Asn Arg Tyr Arg Asp Ile Leu Pro Tyr Asp Ser Thr Arg Val Pro Leu
 195 200 205
 35 Gly Lys Asn Lys Asp Tyr Ile Asn Ala Ser Tyr Ile Arg Ile Val Asn
 210 215 220
 His Glu Glu Glu Tyr Phe Tyr Ile Ala Thr Gln Gly Pro Leu Pro Glu
 225 230 235 240
 Thr Ile Glu Asp Phe Trp Gln Met Val Leu Glu Asn Asn Cys Asn Val
 245 250 255
 45 Ile Ala Met Ile Thr Arg Glu Ile Glu Cys Gly Val Ile Lys Cys Tyr
 260 265 270
 Ser Tyr Trp Pro Ile Ser Leu Lys Glu Pro Leu Glu Phe Glu His Phe
 275 280 285
 50 Ser Val Phe Leu Glu Thr Phe His Val Thr Gln Tyr Phe Thr Val Arg
 290 295 300
 Val Phe Gln Ile Val Lys Lys Ser Thr Gly Lys Ser Gln Cys Val Lys
 305 310 315 320
 His Leu Gln Phe Thr Lys Trp Pro Asp His Gly Thr Pro Ala Ser Ala
 325 330 335
 60 Asp Phe Phe Ile Lys Tyr Val Arg Tyr Val Arg Lys Ser His Ile Thr
 340 345 350
 Gly Pro Leu Leu Val His Cys Ser Ala Gly Val Gly Arg Thr Gly Val
 355 360 365
 65

160

Phe Ile Cys Val Asp Val Val Phe Ser Ala Ile Glu Lys Asn Tyr Ser
370 375 380

5 Phe Asp Ile Met Asn Ile Val Thr Gln Met Arg Lys Gln Arg Cys Gly
385 390 395 400

Met Ile Gln Thr Lys
405

10

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 122 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

20

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

25 Asp Phe Trp Gly Met Met Trp Glu Asn Asn Cys Asn Val Ile Ala Met
1 5 10 15

Ile Thr Arg Glu Ile Glu Gly Gly Val Ile Lys Cys Cys Ser Tyr Trp
20 25 30

30

Pro Val Ser Leu Lys Glu Pro Leu Glu Phe Lys His Phe His Val Leu
35 40 45

35 Leu Glu Asn Phe Gln Ile Thr Gln Tyr Phe Val Ile Arg Ile Phe Gln
50 55 60

Ile Val Lys Lys Ser Thr Gly Lys Ser His Ser Val Lys His Leu Gln
65 70 75 80

40 Phe Ile Lys Trp Pro Asp His Gly Thr Pro Ala Ser Val Asp Phe Phe
85 90 95

Ile Lys Tyr Val Arg Tyr Val Arg Lys Ser His Ile Thr Gly Pro Leu
100 105 110

45

Leu Val His Cys Thr Ala Gly Val Gly Arg
115 120

50

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1274 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55

60 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

65 Met Ala Ala His Glu Ala Ser Ser Leu Tyr Ser Glu Glu Lys Ala Lys
1 5 10 15

161

Leu Leu Arg Glu Met Met Ala Lys Ile Glu Asp Lys Asn Glu Val Leu
 20 25 30
 5
 Asp Gln Phe Met Asp Ser Met Gln Leu Asp Pro Glu Thr Val Asp Asn
 35 40 45
 10
 Leu Asp Ala Tyr Ser His Ile Pro Pro Gln Leu Met Glu Lys Cys Ala
 50 55 60
 Ala Leu Ser Val Arg Pro Asp Thr Val Arg Asn Leu Val Gln Ser Met
 65 70 75 80
 15
 Gln Val Leu Ser Gly Val Phe Thr Asp Val Glu Ala Ser Leu Lys Asp
 85 90 95
 Ile Arg Asp Leu Leu Glu Glu Asp Glu Leu Leu Glu Gln Lys Phe Gln
 100 105 110
 20
 Glu Ala Val Gly Gln Ala Gly Ala Ile Ser Ile Thr Ser Lys Ala Glu
 115 120 125
 Leu Ala Glu Val Arg Arg Glu Trp Ala Lys Tyr Met Glu Val His Glu
 130 135 140
 25
 Lys Ala Ser Phe Thr Asn Ser Glu Leu His Arg Ala Met Asn Leu His
 145 150 155 160
 30
 Val Gly Asn Leu Arg Leu Leu Ser Gly Pro Leu Asp Gln Val Arg Ala
 165 170 175
 Ala Leu Pro Thr Pro Ala Leu Ser Pro Glu Asp Lys Ala Val Leu Gln
 180 185 190
 35
 Asn Leu Lys Arg Ile Leu Ala Lys Val Gln Glu Met Arg Asp Gln Arg
 195 200 205
 Val Ser Leu Glu Gln Gln Leu Arg Glu Leu Ile Gln Lys Asp Asp Ile
 210 215 220
 40
 Thr Ala Ser Leu Val Thr Thr Asp His Ser Glu Met Lys Lys Leu Phe
 225 230 235 240
 45
 Glu Glu Gln Leu Lys Lys Tyr Asp Gln Leu Lys Val Tyr Leu Glu Gln
 245 250 255
 Asn Leu Ala Ala Gln Asp Arg Val Leu Cys Ala Leu Thr Glu Ala Asn
 260 265 270
 50
 Val Gln Tyr Ala Ala Val Arg Arg Val Leu Ser Asp Leu Asp Gln Lys
 275 280 285
 Trp Asn Ser Thr Leu Gln Thr Leu Val Ala Ser Tyr Glu Ala Tyr Glu
 290 295 300
 55
 Asp Leu Met Lys Lys Ser Gln Glu Gly Arg Asp Phe Tyr Ala Asp Leu
 305 310 315 320
 60
 Glu Ser Lys Val Ala Ala Leu Leu Glu Arg Thr Gln Ser Thr Cys Gln
 325 330 335
 Ala Arg Glu Ala Ala Arg Gln Gln Leu Leu Asp Arg Glu Leu Lys Lys
 340 345 350

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	Lys	Pro	Pro	Pro	Arg	Pro	Thr	Ala	Pro	Lys	Pro	Leu	Leu	Pro	Arg	Arg	
			355					360					365				
5	Glu	Glu	Ser	Glu	Ala	Val	Glu	Ala	Gly	Asp	Pro	Pro	Glu	Glu	Leu	Arg	
		370					375					380					
	Ser	Leu	Pro	Pro	Asp	Met	Val	Ala	Gly	Pro	Arg	Leu	Pro	Asp	Thr	Phe	
10	385					390					395					400	
	Leu	Gly	Ser	Ala	Thr	Pro	Leu	His	Phe	Pro	Pro	Ser	Pro	Phe	Pro	Ser	
					405					410					415		
15	Ser	Thr	Gly	Pro	Gly	Pro	His	Tyr	Leu	Ser	Gly	Pro	Leu	Pro	Pro	Gly	
				420					425					430			
	Thr	Tyr	Ser	Gly	Pro	Thr	Gln	Leu	Ile	Gln	Pro	Arg	Ala	Pro	Gly	Pro	
20			435					440					445				
	His	Ala	Met	Pro	Val	Ala	Pro	Gly	Pro	Ala	Leu	Tyr	Pro	Ala	Pro	Ala	
		450					455					460					
25	Tyr	Thr	Pro	Glu	Leu	Gly	Leu	Val	Pro	Arg	Ser	Ser	Pro	Gln	His	Gly	
	465					470					475					480	
	Val	Val	Ser	Ser	Pro	Tyr	Val	Gly	Val	Gly	Pro	Ala	Pro	Pro	Val	Ala	
					485					490					495		
30	Gly	Leu	Pro	Ser	Ala	Pro	Pro	Pro	Gln	Phe	Ser	Gly	Pro	Glu	Leu	Ala	
				500					505					510			
	Met	Ala	Val	Arg	Pro	Ala	Thr	Thr	Thr	Val	Asp	Ser	Ile	Gln	Ala	Pro	
35			515					520					525				
	Ile	Pro	Ser	His	Thr	Ala	Pro	Arg	Pro	Asn	Pro	Thr	Pro	Ala	Pro	Pro	
		530					535					540					
40	Pro	Pro	Cys	Phe	Pro	Val	Pro	Pro	Pro	Gln	Pro	Leu	Pro	Thr	Pro	Tyr	
	545					550					555					560	
	Thr	Tyr	Pro	Ala	Gly	Ala	Lys	Gln	Pro	Ile	Pro	Ala	Gln	His	His	Phe	
					565					570					575		
45	Ser	Ser	Gly	Ile	Pro	Thr	Gly	Phe	Pro	Ala	Pro	Arg	Ile	Gly	Pro	Gln	
				580					585					590			
	Pro	Gln	Pro	His	Pro	Gln	Pro	His	Pro	Ser	Gln	Ala	Phe	Gly	Pro	Gln	
50			595					600					605				
	Pro	Pro	Gln	Gln	Pro	Leu	Pro	Leu	Gln	His	Pro	His	Leu	Phe	Pro	Pro	
		610					615					620					
55	Gln	Ala	Pro	Gly	Leu	Leu	Pro	Pro	Gln	Ser	Pro	Tyr	Pro	Tyr	Ala	Pro	
	625					630					635					640	
	Gln	Pro	Gly	Val	Leu	Gly	Gln	Pro	Pro	Pro	Pro	Leu	His	Thr	Gln	Leu	
					645					650					655		
60	Tyr	Pro	Gly	Pro	Ala	Gln	Asp	Pro	Leu	Pro	Ala	His	Ser	Gly	Ala	Leu	
				660					665					670			
	Pro	Phe	Pro	Ser	Pro	Gly	Pro	Pro	Gln	Pro	Pro	His	Pro	Pro	Leu	Ala	
65			675					680					685				

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	Tyr	Gly	Pro	Ala	Pro	Ser	Thr	Arg	Pro	Met	Gly	Pro	Gln	Ala	Ala	Pro
	690						695					700				
5	Leu	Thr	Ile	Arg	Gly	Pro	Ser	Ser	Ala	Gly	Gln	Ser	Thr	Pro	Ser	Pro
	705					710					715					720
	His	Leu	Val	Pro	Ser	Pro	Ala	Pro	Ser	Pro	Gly	Pro	Gly	Pro	Val	Pro
					725					730					735	
10	Pro	Arg	Pro	Pro	Ala	Ala	Glu	Pro	Pro	Pro	Cys	Leu	Arg	Arg	Gly	Ala
				740					745					750		
15	Ala	Ala	Ala	Asp	Leu	Leu	Ser	Ser	Ser	Pro	Glu	Ser	Gln	His	Gly	Gly
			755					760					765			
	Thr	Gln	Ser	Pro	Gly	Gly	Gly	Gln	Pro	Leu	Leu	Gln	Pro	Thr	Lys	Val
	770						775					780				
20	Asp	Ala	Ala	Glu	Gly	Arg	Arg	Pro	Gln	Ala	Leu	Arg	Leu	Ile	Glu	Arg
	785					790					795					800
	Asp	Pro	Tyr	Glu	His	Pro	Glu	Arg	Leu	Arg	Gln	Leu	Gln	Gln	Glu	Leu
					805					810					815	
25	Glu	Ala	Phe	Arg	Gly	Gln	Leu	Gly	Asp	Val	Gly	Ala	Leu	Asp	Thr	Val
				820					825					830		
30	Trp	Arg	Glu	Leu	Gln	Asp	Ala	Gln	Glu	His	Asp	Ala	Arg	Gly	Arg	Ser
			835					840					845			
	Ile	Ala	Ile	Ala	Arg	Cys	Tyr	Ser	Leu	Lys	Asn	Arg	His	Gln	Asp	Val
	850						855					860				
35	Met	Pro	Tyr	Asp	Ser	Asn	Arg	Val	Val	Leu	Arg	Ser	Gly	Lys	Asp	Asp
	865					870					875					880
	Tyr	Ile	Asn	Ala	Ser	Cys	Val	Glu	Gly	Leu	Ser	Pro	Tyr	Cys	Pro	Pro
					885					890					895	
40	Leu	Val	Ala	Thr	Gln	Ala	Pro	Leu	Pro	Gly	Thr	Ala	Ala	Asp	Phe	Trp
				900					905					910		
45	Leu	Met	Val	His	Glu	Gln	Lys	Val	Ser	Val	Ile	Val	Met	Leu	Val	Ser
		915						920					925			
	Glu	Ala	Glu	Met	Glu	Lys	Gln	Lys	Val	Ala	Arg	Tyr	Phe	Pro	Thr	Glu
	930						935				940					
50	Arg	Gly	Gln	Pro	Met	Val	His	Gly	Ala	Leu	Ser	Leu	Ala	Leu	Ser	Ser
	945					950					955					960
	Val	Arg	Ser	Thr	Glu	Thr	His	Val	Glu	Arg	Val	Leu	Ser	Leu	Gln	Phe
					965					970					975	
55	Arg	Asp	Gln	Ser	Leu	Lys	Arg	Ser	Leu	Val	His	Leu	His	Phe	Pro	Thr
				980					985					990		
60	Trp	Pro	Glu	Leu	Gly	Leu	Pro	Asp	Ser	Pro	Ser	Asn	Leu	Leu	Arg	Phe
		995						1000					1005			
	Ile	Gln	Glu	Val	His	Ala	His	Tyr	Leu	His	Gln	Arg	Pro	Leu	His	Thr
	1010						1015					1020				

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Pro Ile Ile Val His Cys Ser Ser Gly Val Gly Arg Thr Gly Ala Phe
 1025 1030 1035 1040

5 Ala Leu Leu Tyr Ala Ala Val Gln Glu Val Glu Ala Gly Asn Gly Ile
 1045 1050 1055

Pro Glu Leu Pro Gln Leu Val Arg Arg Met Arg Gln Gln Arg Lys His
 1060 1065 1070

10 Met Leu Gln Glu Lys Leu His Leu Arg Phe Cys Tyr Glu Ala Val Val
 1075 1080 1085

Arg His Val Glu Gln Val Leu Gln Arg His Gly Val Pro Pro Pro Cys
 1090 1095 1100

15 Lys Pro Leu Ala Ser Ala Ser Ile Ser Gln Lys Asn His Leu Pro Gln
 1105 1110 1115 1120

20 Asp Ser Gln Asp Leu Val Leu Gly Gly Asp Val Pro Ile Ser Ser Ile
 1125 1130 1135

Gln Ala Thr Ile Ala Lys Leu Ser Ile Arg Pro Pro Gly Gly Leu Glu
 1140 1145 1150

25 Ser Pro Val Ala Ser Leu Pro Gly Pro Ala Glu Pro Pro Gly Leu Pro
 1155 1160 1165

Pro Ala Ser Leu Pro Glu Ser Thr Pro Ile Pro Ser Ser Ser Pro Pro
 1170 1175 1180

30 Pro Leu Ser Ser Pro Leu Pro Glu Ala Pro Gln Pro Lys Glu Glu Pro
 1185 1190 1195 1200

35 Pro Val Pro Glu Ala Pro Ser Ser Gly Pro Pro Ser Ser Ser Leu Glu
 1205 1210 1215

Leu Leu Ala Ser Leu Thr Pro Glu Ala Phe Ser Leu Asp Ser Ser Leu
 1220 1225 1230

40 Arg Gly Lys Gln Arg Met Ser Lys His Asn Phe Leu Gln Ala His Asn
 1235 1240 1245

Gly Gln Gly Leu Arg Ala Thr Arg Pro Ser Asp Asp Pro Leu Ser Leu
 1250 1255 1260

45 Leu Asp Pro Leu Trp Thr Leu Asn Lys Thr
 1265 1270

50

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 493 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

60 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

65 Met Thr Arg Ala Leu Cys Ser Ala Leu Arg Gln Ala Leu Leu Leu Leu
 1 5 10 15

165

	Ala	Ala	Ala	Ala	Glu	Leu	Ser	Pro	Gly	Leu	Lys	Cys	Val	Cys	Leu	Leu	
				20					25					30			
5	Cys	Asp	Ser	Ser	Asn	Phe	Thr	Cys	Gln	Thr	Glu	Gly	Ala	Cys	Trp	Ala	
			35					40					45				
	Ser	Val	Met	Leu	Thr	Asn	Gly	Lys	Glu	Gln	Val	Ile	Lys	Ser	Cys	Val	
			50				55					60					
10	Ser	Leu	Pro	Glu	Leu	Asn	Ala	Gln	Val	Phe	Cys	His	Ser	Ser	Asn	Asn	
						70					75					80	
	Val	Thr	Lys	Thr	Glu	Cys	Cys	Phe	Thr	Asp	Phe	Cys	Asn	Asn	Ile	Thr	
15					85					90					95		
	Leu	His	Leu	Pro	Thr	Ala	Ser	Pro	Asn	Ala	Pro	Lys	Leu	Gly	Pro	Met	
				100					105					110			
20	Glu	Leu	Ala	Ile	Ile	Ile	Thr	Val	Pro	Val	Cys	Leu	Leu	Ser	Ile	Ala	
			115					120					125				
	Ala	Met	Leu	Thr	Val	Trp	Ala	Cys	Gln	Gly	Arg	Gln	Cys	Ser	Tyr	Arg	
25			130				135					140					
	Lys	Lys	Lys	Arg	Pro	Asn	Val	Glu	Glu	Pro	Leu	Ser	Glu	Cys	Asn	Leu	
						150					155					160	
	Val	Asn	Ala	Gly	Lys	Thr	Leu	Lys	Asp	Leu	Ile	Tyr	Asp	Val	Thr	Ala	
30					165					170					175		
	Ser	Gly	Ser	Gly	Ser	Gly	Leu	Pro	Leu	Leu	Val	Gln	Arg	Thr	Ile	Ala	
				180					185					190			
35	Arg	Thr	Ile	Val	Leu	Gln	Glu	Ile	Val	Gly	Lys	Gly	Arg	Phe	Gly	Glu	
			195					200					205				
	Val	Trp	His	Gly	Arg	Trp	Cys	Gly	Glu	Asp	Val	Ala	Val	Lys	Ile	Phe	
			210				215					220					
40	Ser	Ser	Arg	Asp	Glu	Arg	Ser	Trp	Phe	Arg	Glu	Ala	Glu	Ile	Tyr	Gln	
						230					235					240	
	Thr	Val	Met	Leu	Arg	His	Glu	Asn	Ile	Leu	Gly	Phe	Ile	Ala	Ala	Asp	
45					245					250					255		
	Asn	Lys	Asp	Asn	Gly	Thr	Trp	Thr	Gln	Leu	Trp	Leu	Val	Ser	Glu	Tyr	
				260					265					270			
50	His	Glu	Gln	Gly	Ser	Leu	Tyr	Asp	Tyr	Leu	Asn	Arg	Asn	Ile	Val	Thr	
			275					280					285				
	Val	Ala	Gly	Met	Ile	Lys	Leu	Ala	Leu	Ser	Ile	Ala	Ser	Gly	Leu	Ala	
			290				295					300					
55	His	Leu	His	Met	Glu	Ile	Val	Gly	Thr	Gln	Gly	Lys	Pro	Ala	Ile	Ala	
						310					315					320	
	His	Arg	Asp	Ile	Lys	Ser	Lys	Asn	Ile	Leu	Val	Lys	Lys	Cys	Glu	Thr	
60					325					330					335		
	Cys	Ala	Ile	Ala	Asp	Leu	Gly	Leu	Ala	Val	Lys	His	Asp	Ser	Ile	Leu	
				340					345					350			

166

Asn Thr Ile Asp Ile Pro Gln Asn Pro Lys Val Gly Thr Lys Arg Tyr
 355 360 365
 5 Met Ala Pro Glu Met Leu Asp Asp Thr Met Asn Val Asn Ile Phe Glu
 370 375 380
 Ser Phe Lys Arg Ala Asp Ile Tyr Ser Val Gly Leu Val Tyr Trp Glu
 385 390 395 400
 10 Ile Ala Arg Arg Cys Ser Val Gly Gly Ile Val Glu Glu Tyr Gln Leu
 405 410 415
 Pro Tyr Tyr Asp Met Val Pro Ser Asp Pro Ser Ile Glu Glu Met Arg
 420 425 430
 15 Lys Val Val Cys Asp Gln Lys Phe Arg Pro Ser Ile Pro Asn Gln Trp
 435 440 445
 Gln Ser Cys Glu Ala Leu Arg Val Met Gly Arg Ile Met Arg Glu Cys
 450 455 460
 20 Trp Tyr Ala Asn Gly Ala Ala Arg Leu Thr Ala Leu Arg Ile Lys Lys
 465 470 475 480
 25 Thr Ile Ser Gln Leu Cys Val Lys Glu Asp Cys Lys Ala
 485 490

30 (2) INFORMATION FOR SEQ ID NO: 17:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

40 (ix) FEATURE:

(D) OTHER INFORMATION: The letter "Y" stands for C or T.
 The letter "V" stands for A, C or G.
 The letter "R" stands for A or G.
 The letter "N" stands for A, C, G or T.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

50 GAYTTYTGGV RNATGRTNTG GGA 23

55 (2) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:

60 (A) LENGTH: 23 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ix) FEATURE:

167

(D) OTHER INFORMATION: The letter "S" stands for C or G.
The letter "Y" stands for C or T.
The letter "N" stands for A, C, G or T.
The letter "W" stands for A or T.
The letter "R" stands for A or G.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

CGGCCSAYNC CNGCNSWRCA RTG

(2) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: "Xaa" in positions 4 and 6 stand
for an unspecified amino acid.
"Xaa" in position 8 stands for
either Glu or Asp.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asp Phe Trp Xaa Met Xaa Trp Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: "Xaa" in positions 3 and 6 stand
for an unspecified amino acid.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

His Cys Xaa Ala Gly Xaa Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 21:

168

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

10 CACCGTTCGA GTATTCAGA TTGTGAAGAA GTCC 34

15

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

GGACTTCTTC ACAATCTGAA ATACTCGAAC GGTG 34

30

(2) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

CCGTTATGTG AGGAAGAGCC ACATTACAGG ACC 33

45 (2) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 33 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

GGTCCTGTAA TGTGGCTCTT CCTCACATAA CGG 33

60 (2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

169

(A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

GGCATGCATG GAGTATGAAA TGG

23

10

(2) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:

15

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

CGTACATCCC AGATGAGCTC AAGAATAGGG

30

25

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

30

(A) LENGTH: 31 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Ser Trp Pro Pro Ser Gly Thr Ser Ser Lys Met Ser Leu Asp Asp Leu
1 5 10 15

40

Pro Glu Lys Gln Asp Gly Thr Val Phe Pro Ser Ser Leu Leu Pro
20 25 30

45

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

50

(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Tyr Ser Leu Pro Tyr Asp Ser Lys His Gln Ile Arg Asn Ala Ser Asn
1 5 10 15

60

Val Lys His His Asp Ser Ser Ala Leu Gly Val Tyr Ser Tyr
20 25 30

65

170

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

His Thr Leu Gln Ala Asp Ser Tyr Ser Pro Asn Leu Pro Lys Ser Thr
1 5 10 15
Thr Lys Ala Ala Lys Met Met Asn Gln Gln Arg Thr Lys Cys
20 25 30

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The letter "N" stands for A, C, G
or T.
The letter "R" stands for A or G.
The letter "Y" stands for C or T.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

GGNCARTTYG GNGANGTNTG G 21

(2) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The letter "N" stands for A, C, G
or T.
The letter "Y" stands for C or T.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

CAGNGCNGCY TCNGGNGCNG TCCA 24

171

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: "Xaa" in position 5 stands for
either Glu or Asp.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

Gly Gln Phe Gly Xaa Val Trp
1 5

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

Trp Thr Ala Pro Glu Ala Leu Leu
1 5

(2) INFORMATION FOR SEQ ID NO: 34:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

AGTGAGCTTC ATGTTGGCT

19

(2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

172

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:

GGTAGAGGCT GCCATCAG

18

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The letter "N" stands for
deoxythymidylate.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:

GACGATCGGA ATTCGCGAN

19

(2) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:

GACGATCGGA ATTCGCGA

18

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CCCAGCCACA GGCCTTC

17

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

173

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

CCACACCTCC CCAAAGTA

18

(2) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

TGGGAGCGGC CACACTCCGA ATTCGCCCTT

30

(2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GCCTGCGTGC GAAGATG

17

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CTTCGAGGGC ACAGAGCC

18

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

5 ATGGAGCCGT TCCTCAGGAG G 21

(2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

20 TCACCCAGCT TCCTCCAAG G 21

(2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 21 base pairs
30 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

35 AGGCCAACTG GAAGCTGATC C 21

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

40 (A) LENGTH: 20 base pairs
45 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

50 GCTGGAGCCC AGAGCGTTGG 20

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

55 (A) LENGTH: 8 amino acids
60 (B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

65 (ix) FEATURE:

175

(D) OTHER INFORMATION: "Xaa" in position 6 stands
for an unspecified amino acid.

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

His Arg Asp Leu Arg Xaa Ala Asn
1 5

10

(2) INFORMATION FOR SEQ ID NO: 48:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

25 (D) OTHER INFORMATION: "Xaa" in positions 6 stands
for an unspecified amino acid.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

30 His Arg Asp Leu Ala Xaa Arg Asn
1 5

35 (2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

45

TCGCCAAGGA GATCCAGACA C

21

50 (2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

60

GAAGTCAGCC ACCTTGCAGG C

21

176

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

GGATCCCCGG ACC

(2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

Met Arg Gly Ser His His His His His His
1 5 10

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

ATGAGAGGAT CGCATCACCA TCACCATCAC

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

177

(D) OTHER INFORMATION: "Xaa" in positions 4 and 6 stand
for an unspecified amino acid.
"Xaa" in position 8 stands for
either Glu or Asp.

5

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

Asp Phe Trp Xaa Met Xaa Trp Xaa
1 5

10

(2) INFORMATION FOR SEQ ID NO: 55:

15

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser
1 5 10

30

(2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

45

His Cys Ser Ala Gly
1 5

50

(2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55

(ii) MOLECULE TYPE: peptide

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

Met Ser Ser Pro Arg Lys Val Arg Gly Lys Thr Gly Arg Asp Asn Asp
1 5 10 15

65

178

Glu Glu Glu Gly Asn Ser Gly Asn Leu Asn Leu Arg Asn
 20 25

5

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:

20 Ser Pro Val Leu Ser Gly Ser Ser Arg Leu Ser Lys Asp Thr Glu Thr
 1 5 10 15
 Ser Val Ser Glu Lys Glu Leu Thr Gln Leu Ala Gln Ile
 20 25

25

(2) INFORMATION FOR SEQ ID NO: 59:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 29 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:

40 Trp Asp Val Ser Asp Arg Ser Leu Arg Asn Arg Trp Asn Ser Met Asp
 1 5 10 15
 Ser Glu Thr Ala Gly Pro Ser Lys Thr Val Ser Pro Val
 20 25

45

(2) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 26 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

55

(ix) FEATURE:

60 (D) OTHER INFORMATION: The letter "Y" stands for C or T.
 The letter "H" stands for A, C or T.
 The letter "M" stands for A or C.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:

65 ATCCCCGGCT CTGAYTAYAT HMAYGC

26

(2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: "Xaa" in position 8 stands for
either Asn or His.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 61:

Ile Pro Gly Ser Asp Tyr Ile Xaa Ala
1 5

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:

Met Glu Glu Leu Gln Asp Tyr Glu Asp Met Met Glu Glu Asn
1 5 10

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:

Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
1 5 10 15
Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp
20 25 30

180

(2) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

15 (ix) FEATURE:

(D) OTHER INFORMATION: "Xaa" in positions 6 and 7 stand
for an unspecified amino acid.

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:

His Arg Asp Leu Lys Xaa Xaa Asn
1 5

25

(2) INFORMATION FOR SEQ ID NO: 65:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 23 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear

(ix) FEATURE:

(D) OTHER INFORMATION: The letter "R" stands for A or G.
The letter "N" stands for Inosine.

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

GARRARGTNG CNGTNAARRT NTT

23

45

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

50 (A) LENGTH: 29 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

55 (ix) FEATURE:

(D) OTHER INFORMATION: The letter "R" stands for A or G.
The letter "N" stands for Inosine.
The letter "K" stands for G or T.
The letter "M" stands for A or C.
The letter "Y" stands for C or T.

60

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

65 TTRATRTCNC KRTGNGMNAT NGMNGGYTT

29

181

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: "Xaa" in position 2 stands for Lys or
Glu. "Xaa" in position 7 stands for
Val or Ile.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

Glu Xaa Val Ala Val Lys Xaa Phe
1 5

(2) INFORMATION FOR SEQ ID NO: 68:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(D) OTHER INFORMATION: "Xaa" in position 3 stands for Ala or
Ser. "Xaa" in position 5 stands for
Ala or Ser.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

Lys Pro Xaa Ile Xaa His Arg Asp Ile Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

AACTTTGGCT GGTATCTGAA TATC

24

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

CCTTGTGTAC CAACAATCTC CATA

24

(2) INFORMATION FOR SEQ ID NO: 71:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

CTCCAGAGAT GAGAGATCTT GG

22

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

TTCCAGCCAC GGTCATATG TT

22

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:

CTTCGAAAGC TTGAAATCGG TACCATCGAT TCTAGAGTTA ACTTCGAA

48

(2) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 47 base pairs
(B) TYPE: nucleic acid

183

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:

5

CTCTAGAACG CGTTAAGGCG CGCCAATATC GATGAATTCT TCGAAGC

47

10

15 (2) INFORMATION FOR SEQ ID NO: 75:

(i) SEQUENCE CHARACTERISTICS:

20

(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

His Cys Ser Ser Gly
1 5

30

(2) INFORMATION FOR SEQ ID NO: 76:

35

(i) SEQUENCE CHARACTERISTICS:

40

(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:
Tyr Arg Lys Lys Lys Arg Pro Asn Val Glu Glu Pro Leu

Claims

1. An isolated, enriched or purified nucleic acid molecule encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide.

5

2. The nucleic acid molecule of claim 1

(a) having the sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8;

10

(b) that hybridizes under highly stringent conditions to the sequence set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:8;

(c) that encodes a polypeptide having the amino acid sequence set forth in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16; or

(d) that encodes a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain polypeptide.

20

3. The nucleic acid molecule of claim 1 where the nucleic acid molecule is isolated, enriched, or purified from a human.

25 4. The nucleic acid molecule of claim 2 wherein said molecule comprises a

(a) nucleotide sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:9 and is lacking at least one of the following segments of amino acid residues: 1 - 48, 49 - 294, 295 - 807;

30 (b) nucleotide sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:10 and is lacking

185

at least one of the following segments of amino acid residues:

1 - 55, 56 - 109, 120 - 212, 230 - 480, 481 - 488;

(c) nucleotide sequence that encodes a polypeptide having the amino acid sequence of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13 or SEQ. ID. NO:14, and is lacking at least one of the following segments of amino acid residues;

(d) nucleotide sequence that encodes a polypeptide having the amino acid sequence of SEQ. ID. NO: 15 and is lacking at least one of the following segments of amino acid residues: 1 - 857, 353 - 777, 858 - 1096, 1097 - 1274, 1101 - 1214;

(e) encodes a polypeptide having the amino acid sequence of SEQ ID NO:16 and lacking at least one of the following segments of amino acid residues: 1-25, 26-113, 114-493, 193-483; or

(f) hybridizes under stringent conditions to the nucleotide sequence of (a)-(f).

5. A nucleic acid probe for the detection of nucleic acid encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide in a sample.

6. The probe of claim 5, wherein said polypeptide comprises at least 6 contiguous amino acids of the amino acid sequence shown in SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16.

7. A isolated, enriched or purified nucleic acid sequence encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain polypeptide.

8. A recombinant nucleic acid molecule encoding a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide or a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain polypeptide, and a vector or promoter effective to initiate transcription in a host cell.

9. A recombinant nucleic acid molecule encoding

(a) a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide; or

(b) a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain polypeptide fused to a heterologous polypeptide.

10. A recombinant cell comprising a nucleic acid molecule encoding

(a) a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide;

(b) a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain polypeptide; or

(c) a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide or PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 domain polypeptide fused to a heterologous polypeptide.

11. An isolated, enriched or purified PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide.

12. An isolated, enriched or purified PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 domain polypeptide.

13. The PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 polypeptide of claim 11 wherein said polypeptide comprises

(a) an amino acid sequence of SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, or SEQ ID NO:16;

(b) an amino acid sequence encoded by a nucleic acid molecule that hybridizes under highly stringent conditions to the nucleic acid molecule of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or
5 SEQ ID NO:8; or

(c) a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain polypeptide.

14. An antibody having specific binding affinity to a
10 PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide or a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 domain polypeptide.

15. A hybridoma which produces an antibody having
15 specific binding affinity to a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide.

16. A method for identifying a substance capable of
modulating PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity
20 comprising the steps of:

(a) contacting a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide with a test substance, and

(b) determining whether said substance alters the activity of said polypeptide.

25
17. A method for identifying a substance capable of modulating PTP04, SAD, PTP05, PTP10, ALP, or ALK-7 activity in a cell comprising the steps of:

(a) expressing a PTP04, a SAD, a PTP05, a PTP10, an
30 ALP, or an ALK-7 polypeptide in a cell,

(b) adding a test substance to said cells, and

(c) monitoring a change in cell phenotype, cell proliferation, cell differentiation, PTP04, SAD, PTP05, PTP10,

ALP, or ALK-7 catalytic activity, or the interaction between a PTP04, a SAD, a PTP05, a PTP10, an ALP, or an ALK-7 polypeptide and a natural binding partner.

5 18. A method of preventing or treating an abnormal condition by administering to a patient in need of such treatment a compound that modulates the function of a PTP04, a SAD, a PTP05, a PTP10, or an ALP polypeptide in vitro.

10 19. The method of claim 18, wherein said abnormal condition involves abnormality in PTP04, SAD, PTP05, PTP10, or ALP signal transduction pathway.

15 20. The method of claim 19, wherein said abnormal condition is cancer.

20 21. A method of promoting neuronal survival by administering to a patient in need of such treatment a substance which modulates an activity of ALK-7 in vitro.

22. A method for identifying modulators of protein activity comprising the steps of:

- a) contacting a protein with a natural binding partner, thereby forming a captured protein;
- 25 b) contacting said captured protein with a test compound;
- c) measuring said protein activity; and
- d) comparing said protein activity with the activity of a control protein to determine the extent of
- 30 modulation, wherein said control protein has the same amino acid sequence of the protein of step a) without said natural binding partner.

23. The method of claim 22, wherein said method utilizes non-radioactive reagents.

24. The method of claim 23, wherein said protein is not a fusion protein.

25. The method of claim 24, wherein said protein is not a GST-fusion protein.

26. The method of claim 25, wherein said protein is an enzyme, a receptor enzyme, or a non-receptor enzyme.

27. The method of claim 26, wherein said protein is a protein kinase.

28. The method of claim 27, wherein said protein kinase is a protein tyrosine kinase.

29. The method of claim 28, wherein said protein tyrosine kinase is Zap70 or Syk.

30. The method of claim 26, wherein said protein is a protein phosphatase.

31. The method of claim 30, wherein said protein phosphatase is a protein tyrosine phosphatase.

32. The method of claim 31, wherein said protein tyrosine phosphatase is PTP04, SAD, PTP05, PTP10, ALP, or ALK-7.

33. The method of claim 23, wherein said natural binding partner is capable of binding to a solid support.

34. The method of claim 33, wherein said natural binding partner is a peptide.

35. The method of claim 34, wherein said peptide
5 comprises a phosphopeptide.

36. The method of claim 35, wherein said phosphopeptide comprises an ITAM motif.

10 37. The method of claim 33, wherein said natural binding partner comprises a lipid.

38. The method of claim 33, wherein said solid support
15 comprises well plate, glass beads, or resin.

39. The method of claim 23, wherein said activity is autocatalytic activity, catalytic turnover of substrate, or binding of a second natural binding partner.

20 40. The method of claim 23, further comprising the step of contacting said capture protein with one or more components of the group consisting of a substrate, a second natural binding partner, and an antibody.

25 41. The method of claim 23, further comprising the step of lysing cells containing said protein prior to step (a).

42. A kit for the identification of modulators of non-receptor enzyme activity comprising:

- 30
- a) a natural binding partner;
 - b) a solid support; and
 - c) a binding agent.

43. The kit of claim 42, wherein said binding agent is selected from the group consisting of a substrate, a second natural binding partner, and an antibody.

5 44. The kit of claim 43, wherein said natural binding partner is a peptide.

45. The kit of claim 44, wherein said peptide is a phosphopeptide.

10

46. The kit of claim 45, wherein said phosphopeptide comprises an ITAM motif.

15 47. The kit of claim 42, wherein said natural binding partner comprises a lipid.



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/54, 15/55, 9/12, 9/16, C07K 14/705, C12N 15/11, C07K 16/40, 16/28, C12N 5/12, 15/62, C12Q 1/42, 1/48	A3	(11) International Publication Number: WO 98/49317 (43) International Publication Date: 5 November 1998 (05.11.98)																		
(21) International Application Number: PCT/US98/08439 (22) International Filing Date: 27 April 1998 (27.04.98) (30) Priority Data: <table border="0" style="width: 100%;"> <tr> <td style="width: 40%;">60/044,428</td> <td style="width: 30%;">28 April 1997 (28.04.97)</td> <td style="width: 30%;">US</td> </tr> <tr> <td>60/047,222</td> <td>20 May 1997 (20.05.97)</td> <td>US</td> </tr> <tr> <td>60/049,756</td> <td>11 June 1997 (11.06.97)</td> <td>US</td> </tr> <tr> <td>60/049,477</td> <td>11 June 1997 (11.06.97)</td> <td>US</td> </tr> <tr> <td>60/049,914</td> <td>18 June 1997 (18.06.97)</td> <td>US</td> </tr> <tr> <td>60/063,595</td> <td>23 October 1997 (23.10.97)</td> <td>US</td> </tr> </table> (71) Applicant (for all designated States except US): SUGEN, INC. [US/US]; 351 Galveston Drive, Redwood City, CA 94063 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): PLOWMAN, Greg, D. [US/US]; 4 Honeysuckle Lane, San Carlos, CA 94070 (US). CLARY, Douglas [US/US]; 164 Midcrest Way, San Francisco, CA 94131 (US). JALLAL, Bahija [MA/US]; 101 O'Keefe Street, Melo Park, CA 94025 (US). PELES, Elior [IL/IL]; Hanasi Harishon 51, 76303 Rhovot (IL). ONRUST, Susan [US/NZ]; 6 Summit Drive, Mt. Albert, Auckland 3 (NZ). MARKBY, Dave [US/US]; Apartment A, 477 Burnett		60/044,428	28 April 1997 (28.04.97)	US	60/047,222	20 May 1997 (20.05.97)	US	60/049,756	11 June 1997 (11.06.97)	US	60/049,477	11 June 1997 (11.06.97)	US	60/049,914	18 June 1997 (18.06.97)	US	60/063,595	23 October 1997 (23.10.97)	US	Avenue, San Francisco, CA 94131 (US). COURTNEIDGE, Sara, A. [GB/US]; 1408 Alvarado Avenue, Burlingame, CA 94010 (US). APP, Harald [DE/US]; 630 27th Street, San Francisco, CA 94131 (US). HUI, Terance, H. [CN/US]; 363 Skyline Drive, Daly City, CA 94015 (US). (74) Agents: WARBURG, Richard, J. et al.; Lyon & Lyon LLP, Suite 4700, 633 West Fifth Street, Los Angeles, CA 90071-2066 (US). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> (88) Date of publication of the international search report: 25 February 1999 (25.02.99)
60/044,428	28 April 1997 (28.04.97)	US																		
60/047,222	20 May 1997 (20.05.97)	US																		
60/049,756	11 June 1997 (11.06.97)	US																		
60/049,477	11 June 1997 (11.06.97)	US																		
60/049,914	18 June 1997 (18.06.97)	US																		
60/063,595	23 October 1997 (23.10.97)	US																		
(54) Title: DIAGNOSIS AND TREATMENT OF PHOSPHATASE OR KINASE-RELATED DISORDERS (57) Abstract <p>The present invention relates to phosphatases and kinases, nucleic acids encoding such polypeptides, cells, tissues and animals containing such nucleic acids, antibodies to such polypeptides, assays utilizing such polypeptides, and methods relating to all of the foregoing. Methods for treatment, diagnosis, and screening are provided for phosphatase- or kinase-related diseases or conditions characterized by an abnormal interaction between a phosphatase or a kinase and its binding partner.</p>																				

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INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 98/08439

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/54 C12N15/55 C12N9/12 C12N9/16 C07K14/705 C12N15/11 C07K16/40 C07K16/28 C12N5/12 C12N15/62 C12Q1/42 C12Q1/48					
According to International Patent Classification (IPC) or to both national classification and IPC					
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12N C07K C12Q					
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched					
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)					
C. DOCUMENTS CONSIDERED TO BE RELEVANT					
Category	Citation of document, with indication, where appropriate, of the relevant passages				Relevant to claim No.
X A	WO 96 34985 A (SUGEN INC) 7 November 1996 see page 5, line 3 - page 6, line 9 see page 15, line 17 - line 24 ---				5,6 1-4,7-17
X	EMBL database entry HS1185621; accessionnumber AA281242; 4. April 1997; Robert Strausberg: 'National Cancer Institute, Cancer Genome Anatomy Project.' XP002076843 see abstract ---				2,4-6
A	WO 95 06735 A (LUDWIG INST CANCER RES ;GONEZ LEONEL JORGE (SE); SARAS JAN (SE); C) 9 March 1995 see page 43, line 5 - page 44, line 25; examples 1-8 --- -/--				1-20, 22-26, 30-41
<input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.					
° Special categories of cited documents : *A* document defining the general state of the art which is not considered to be of particular relevance *E* earlier document but published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art. *&* document member of the same patent family					
Date of the actual completion of the international search 17 September 1998			Date of mailing of the international search report 21.12.98		
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016			Authorized officer Mandl, B		

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/08439

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>AOKI N. ET AL.: "THE NOVEL PROTEIN-TYROSINE PHOSPHATASE PTP20 IS A POSITIVE REGULATOR OF PC12 CELL NEURONAL DIFFERENTIATION" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 46, 15 November 1996, pages 29422-29426, XP002053901 see 'Experimental Procedures'</p>	1-17
A	<p>SARAS J. ET AL.: "CLONING AND CHARACTERIZATION OF PTPL1, A PROTEIN TYROSINE PHOSPHATASE WITH SIMILARITIES TO CYTOSKELETAL-ASSOCIATED PROTEINS." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 269, no. 39, 1994, pages 24082-24089, XP002076840 see the whole document, especially 'Materials and Methods'</p>	1-17
A	<p>MATTHEWS R. J. ET AL.: "Characterization of hematopoietic intracellular protein tyrosine phosphatases: Description of a phosphatase containing an SH2 domain and another enriched in proline-, glutamic acid-, serine-, and threonine-rich sequences." MOLECULAR AND CELLULAR BIOLOGY, vol. 12, no. 5, 1992, pages 2396-2405, XP002076841 see the whole document</p>	1-17
A	<p>MAEKAWA K. ET AL.: "MOLECULAR CLONING OF A NOVEL PROTEIN-TYROSINE PHOSPHATASE CONTAINING A MEMBRANE-BINDING DOMAIN AND glgf REPEATS." FEBS LETTERS, vol. 337, 1994, pages 200-206, XP002076842 see 'Materials and Methods'</p>	1-17
A	<p>WO 93 10242 A (COLD SPRING HARBOR LAB) 27 May 1993 see page 6, line 21 - page 7, line 23</p>	22-26, 30-41

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/08439

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: 18-20
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
Claims 18-20 could not be searched because the 'compound that modulates the function of a PTP04, a SAD, a PTP05, a PTP10, or an ALP polypeptide' was not precisely specified.
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-20, 22-26 and 30-41 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/08439

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-20,22-26 and 30-41 (all partially)

A PTP04 tyrosine phosphatase and the nucleic acid encoding it; a nucleic acid probe for the detection of said nucleic acid; a nucleic acid molecule encoding a PTP04 tyrosine phosphatase and a vector or a promoter; a nucleic acid molecule encoding a PTP04 tyrosine phosphatase fused to a heterologous polypeptide; a recombinant cell comprising a nucleic acid encoding a PTP04 tyrosine phosphatase; an antibody specific for said PTP04 tyrosine phosphatase and a hybridoma cell that produces said antibody; a method for identifying a substance capable of modulating the activity of the PTP04 tyrosine phosphatase; a method for preventing or treating an abnormal condition by administering a compound that modulates the function of a PTP04 tyrosine phosphatase; a method of identifying modulators of protein activity as far as they refer to a PTP04 tyrosine phosphatase.

2. Claims: 1-20,22-28 and 33-41 (all partially)

A SAD tyrosine kinase and the nucleic acid encoding it; a nucleic acid probe for the detection of said nucleic acid; a nucleic acid molecule encoding a SAD tyrosine kinase and a vector or a promoter; a nucleic acid molecule encoding a SAD tyrosine kinase fused to a heterologous polypeptide; a recombinant cell comprising a nucleic acid encoding a SAD tyrosine kinase; an antibody specific for said SAD tyrosine kinase and a hybridoma cell that produces said antibody; a method for identifying a substance capable of modulating the activity of the SAD tyrosine kinase; a method for preventing or treating an abnormal condition by administering a compound that modulates the function of a SAD tyrosine kinase; a method of identifying modulators of protein activity as far as they refer to a SAD tyrosine kinase.

3. Claims: 1-20,22-26 and 30-41 (all partially)

A PTP05 tyrosine phosphatase and the nucleic acid encoding it; a nucleic acid probe for the detection of said nucleic acid; a nucleic acid molecule encoding a PTP05 tyrosine phosphatase and a vector or a promoter; a nucleic acid molecule encoding a PTP05 tyrosine phosphatase fused to a heterologous polypeptide; a recombinant cell comprising a nucleic acid encoding a PTP05 tyrosine phosphatase; an antibody specific for said PTP05 tyrosine phosphatase and a hybridoma cell that produces said antibody; a method for identifying a substance capable of modulating the activity of the PTP05 tyrosine phosphatase; a method for preventing or treating an abnormal condition by administering a

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/08439

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

compound that modulates the function of a PTP05 tyrosine phosphatase; a method of identifying modulators of protein activity as far as they refer to a PTP05 tyrosine phosphatase.

4. Claims: 1-20, 22-26 and 30-41 (all partially)

A PTP10 tyrosine phosphatase and the nucleic acid encoding it; a nucleic acid probe for the detection of said nucleic acid; a nucleic acid molecule encoding a PTP10 tyrosine phosphatase and a vector or a promoter; a nucleic acid molecule encoding a PTP10 tyrosine phosphatase fused to a heterologous polypeptide; a recombinant cell comprising a nucleic acid encoding a PTP10 tyrosine phosphatase; an antibody specific for said PTP10 tyrosine phosphatase and a hybridoma cell that produces said antibody; a method for identifying a substance capable of modulating the activity of the PTP10 tyrosine phosphatase; a method for preventing or treating an abnormal condition by administering a compound that modulates the function of a PTP10 tyrosine phosphatase; a method of identifying modulators of protein activity as far as they refer to a PTP10 tyrosine phosphatase.

5. Claims: 1-20, 22-26 and 30-41 (all partially)

An ALP tyrosine phosphatase and the nucleic acid encoding it; a nucleic acid probe for the detection of said nucleic acid; a nucleic acid molecule encoding an ALP tyrosine phosphatase and a vector or a promoter; a nucleic acid molecule encoding an ALP tyrosine phosphatase fused to a heterologous polypeptide; a recombinant cell comprising a nucleic acid encoding an ALP tyrosine phosphatase; an antibody specific for said ALP tyrosine phosphatase and a hybridoma cell that produces said antibody; a method for identifying a substance capable of modulating the activity of the ALP tyrosine phosphatase; a method for preventing or treating an abnormal condition by administering a compound that modulates the function of an ALP tyrosine phosphatase; a method of identifying modulators of protein activity as far as they refer to a PTP10 tyrosine phosphatase.

6. Claims: 21 (complete) and 1-20, 22-27, 33-41 (partially)

An ALK-7 type I receptor ser/thr kinase and the nucleic acid encoding it; a nucleic acid probe for the detection of said nucleic acid; a nucleic acid molecule encoding an ALK-7 type I receptor ser/thr kinase and a vector or a promoter; a nucleic acid molecule encoding an ALK-7 type I receptor ser/thr kinase fused to a heterologous polypeptide; a recombinant cell comprising a nucleic acid encoding an ALK-7 type I receptor ser/thr kinase; an antibody specific for

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US 98/08439

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

said ALK-7 type I receptor ser/thr kinase and a hybridoma cell that produces said antibody; a method for identifying a substance capable of modulating the activity of the ALK-7 type I receptor ser/thr kinase; a method of promoting neuronal survival by administering a compound that modulates the activity of an ALK-7 type I receptor ser/thr kinase; a method of identifying modulators of protein activity as far as they refer to an ALK-7 type I receptor ser/thr kinase.

7. Claims: 29,42-47 (complete) and 22-28,30-41 (partially)

A method for identifying modulators of protein activation as far as they do not refer to the following phosphatases or kinases: PTP04, PTP05, PTP10, SAD, ALP and ALK-7; and a kit for the identification of modulators of non-receptor enzyme activity.

INTERNATIONAL SEARCH REPORT

Information on patent family members

In. ational Application No

PCT/US 98/08439

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